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Fun with High Throughput Toxicokinetics

Webinar Presentation to CalEPA
Office of Environmental Health Hazard Assessment

June 12, 2017

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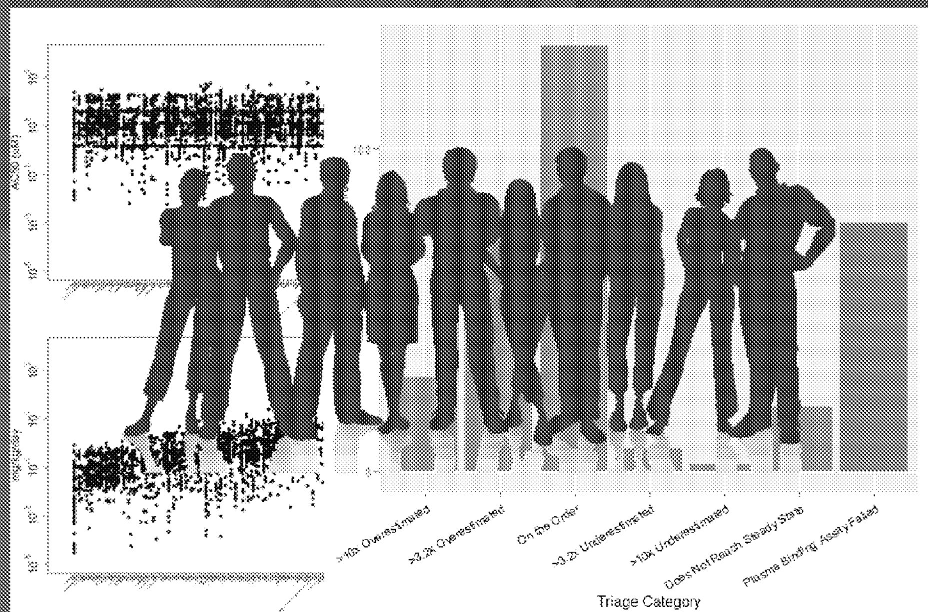

Figure includes image from Thinkstock

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Introduction

- Toxicokinetics (TK) provides a bridge between toxicity and exposure assessment by predicting tissue concentrations due to exposure
  - However traditional TK methods are resource intensive
- Relatively high throughput TK (HTTK) methods have been used by the pharmaceutical industry to determine range of efficacious doses and to prospectively evaluate success of planned clinical trials (Jamei, *et al.*, 2009; Wang, 2010)
  - A key application of HTTK has been "reverse dosimetry" (also called Reverse TK or RTK)
  - RTK can approximately convert *in vitro* HTS results to daily doses needed to produce similar levels in a human for comparison to exposure data (starting off with Rotroff, *et al.*, 2010)
- A new EPA open source R package ("httk") is freely available on CRAN allows RTK and other statistical analyses of 553 chemicals (more coming)

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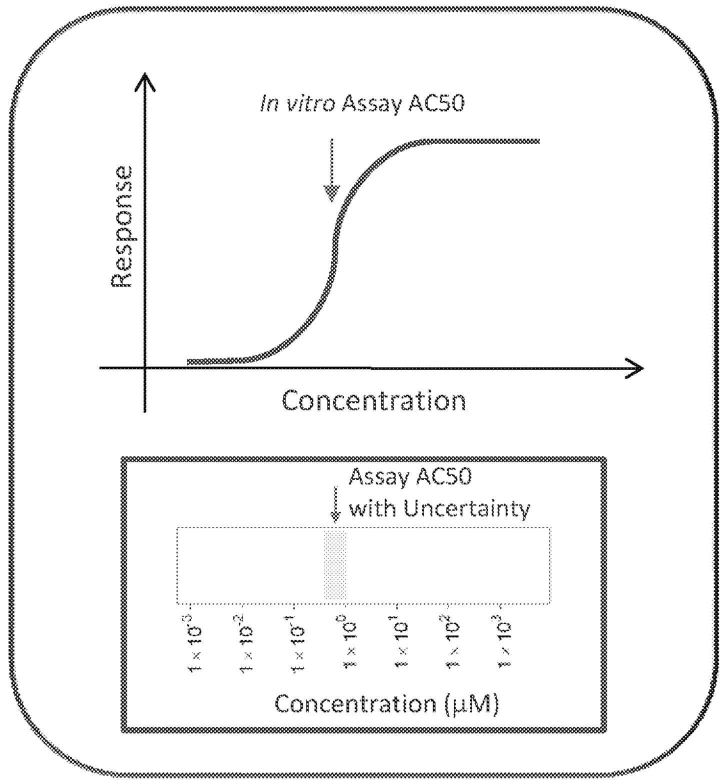
High-Throughput Bioactivity

- **Tox21**: Examining >8,000 chemicals using ~50 assays intended to identify interactions with biological pathways (Schmidt, 2009)

- **ToxCast**: For a subset (>2000) of Tox21 chemicals ran >1100 additional assays (Judson *et al.*, 2010)

- Most assays conducted in dose-response format (identify 50% activity concentration – AC50 – and efficacy if data described by a Hill function, Filer *et al.*, 2016)

- All data is public: http://comptox.epa.gov/



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in vitro – *in vivo* Concordance

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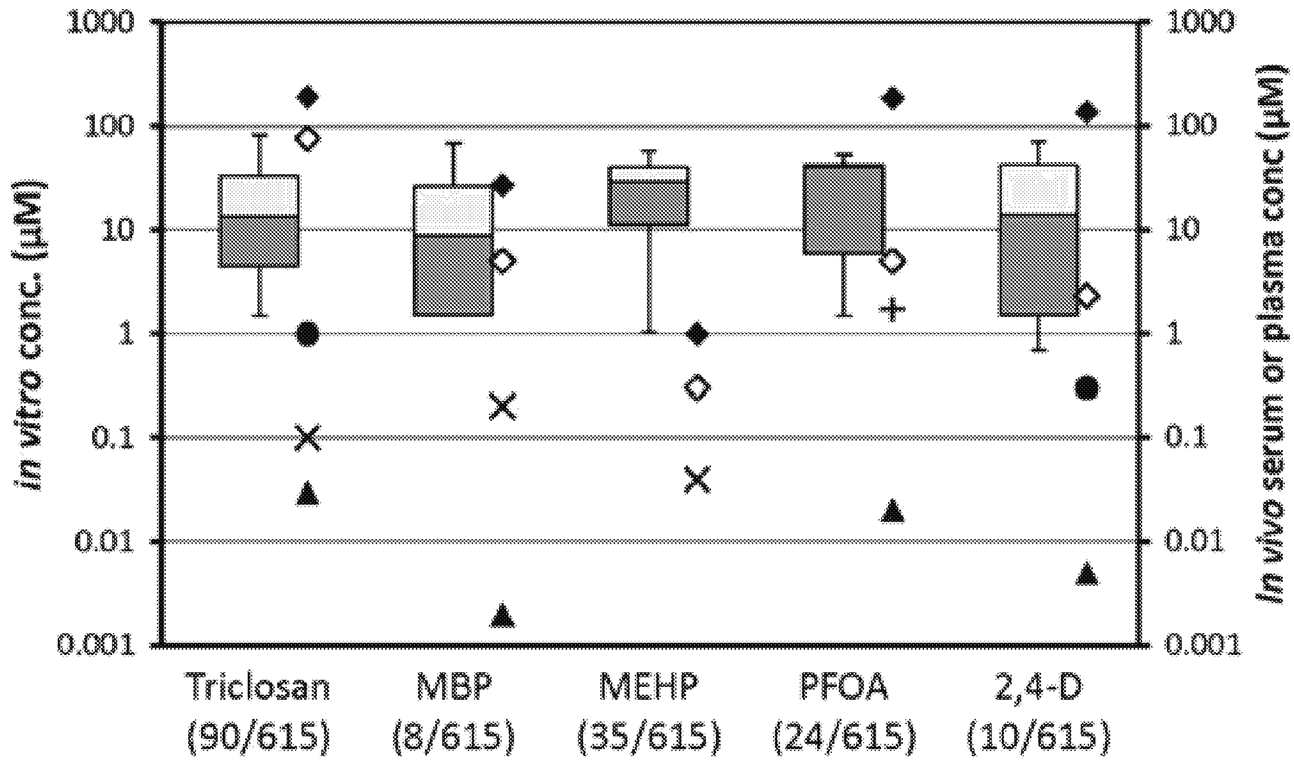


◆ estimated or measured average concentrations associated with the LOAEL in animal studies

◇ NOAEL in animal studies

● Humans with chronic exposure reference values (solid circles)

X Volunteers using products containing the chemical

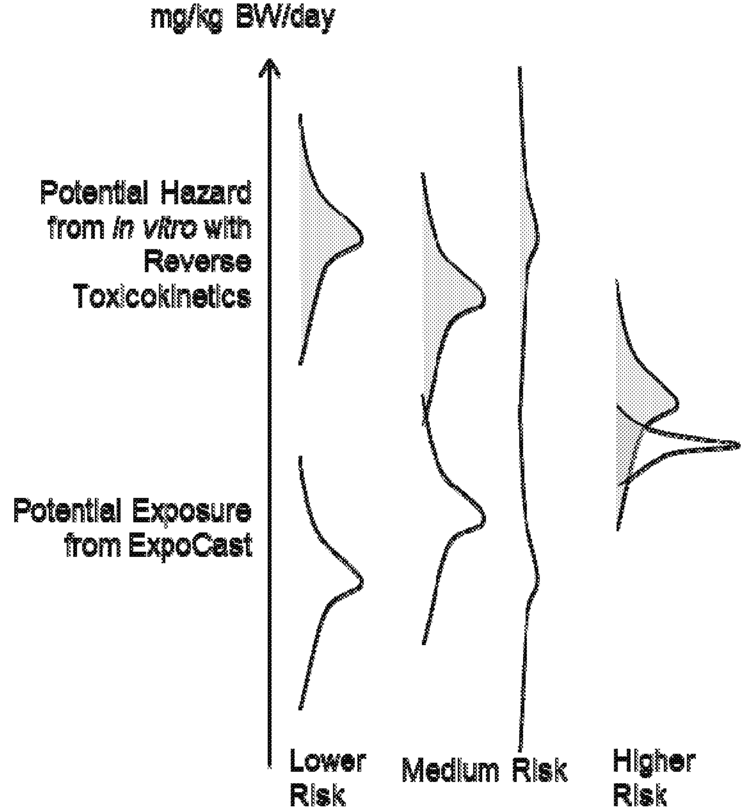
+ Biomonitored occupational populations

▲ General populations

Aylward and Hays (2011)
Journal of Applied Toxicology **31** 741-751

High Throughput Risk Prioritization

- **High throughput risk prioritization** relies on three components:
  1. high throughput **hazard** characterization
  2. high throughput **exposure** forecasts
  3. high throughput **toxicokinetics** (*i.e.*, dosimetry)
- While advances have been made in toxicity and exposure screening, TK methods applicable to 100s of chemicals are needed



see Wetmore et al. (2015)

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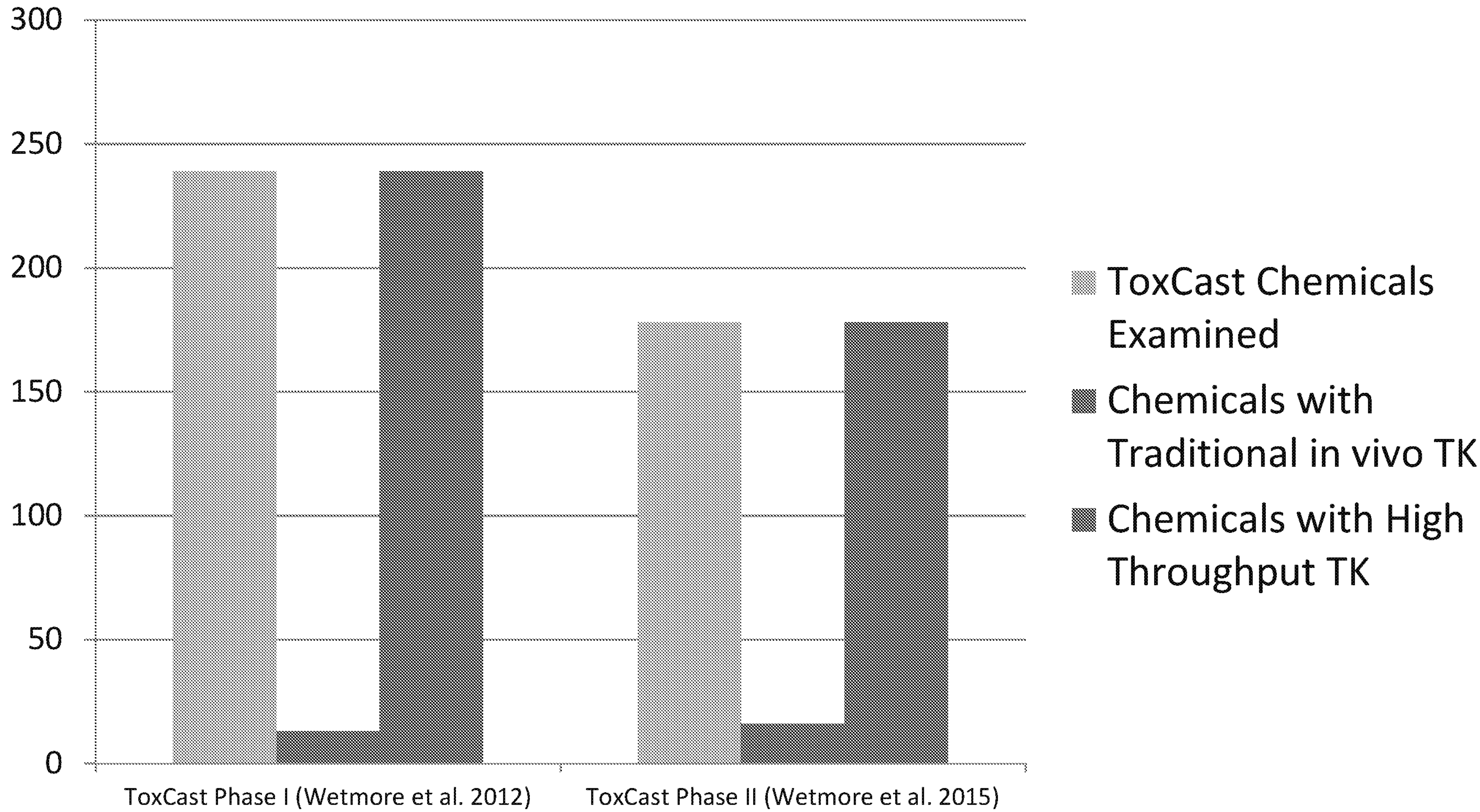
The Need for *In Vitro* Toxicokinetics



- Studies like Wetmore et al. (2012, 2015), address the need for TK data using *in vitro* methods

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In Vitro - *In Vivo* Extrapolation (IVIVE)

Definition:
IVIVE is the utilization of *in vitro* experimental data to predict phenomena in vivo

- IVIVE-PK/TK (Pharmacokinetics/Toxicokinetics):
  - Fate of molecules/chemicals in body
  - Considers absorption, distribution, metabolism, excretion (ADME)
  - Uses empirical PK and physiologically-based (PBPK) modeling

- IVIVE-PD/TD (Pharmacodynamics/Toxicodynamics):
  - Effect of molecules/chemicals at biological target *in vivo*
  - Assay design/selection important
  - Perturbation as adverse/therapeutic effect, reversible/ irreversible

- Both contribute to predict *in vivo* effects

Slide from Barbara Wetmore

High Throughput Toxicokinetics (HTTK)

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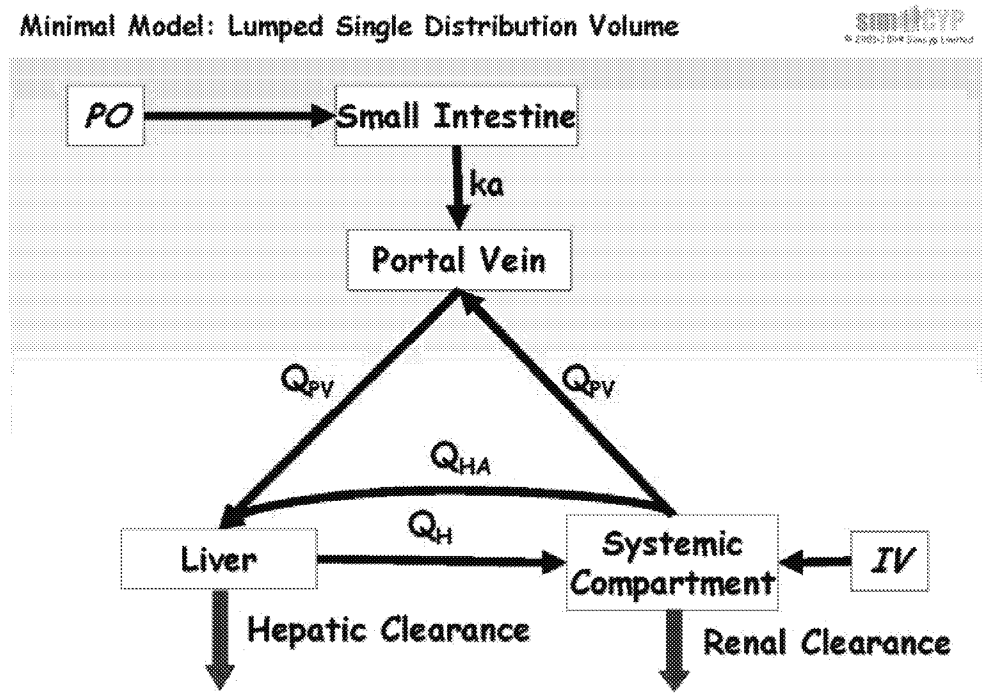
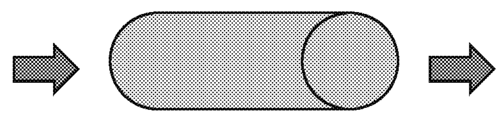
Jamei *et al.* (2009)



- *In vitro* plasma protein binding and metabolic clearance assays allow approximate hepatic and renal clearances to be calculated
- At steady state this allows conversion from concentration to administered dose
- 100% bioavailability assumed

$$C_{ss} = \frac{\text{oral dose rate}}{(\mathrm{GFR} * \mathrm{F_{ub}}) + \left(\mathrm{Q_l} * \mathrm{F_{ub}} * \frac{Cl_{int}}{\mathrm{Q_l} + \mathrm{F_{ub}} * Cl_{int}}\right)}$$

Oral dose in (mg/kg/day)



Sum of hepatic and renal clearance (mg/kg/day)

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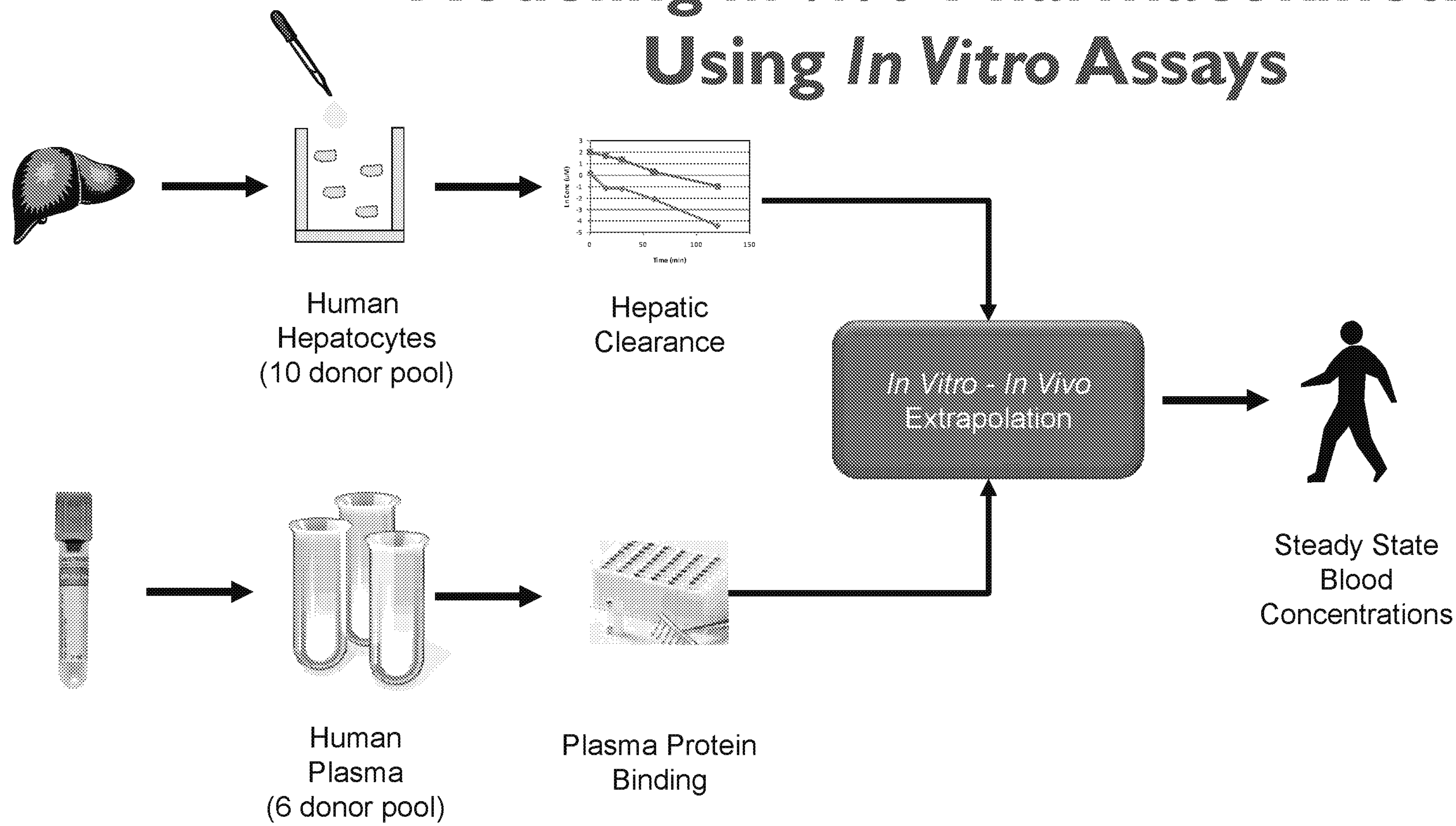
IVIVE in a High-Throughput Environment - Modeling *In Vivo* Pharmacokinetics Using *In Vitro* Assays



Slide from Barbara Wetmore

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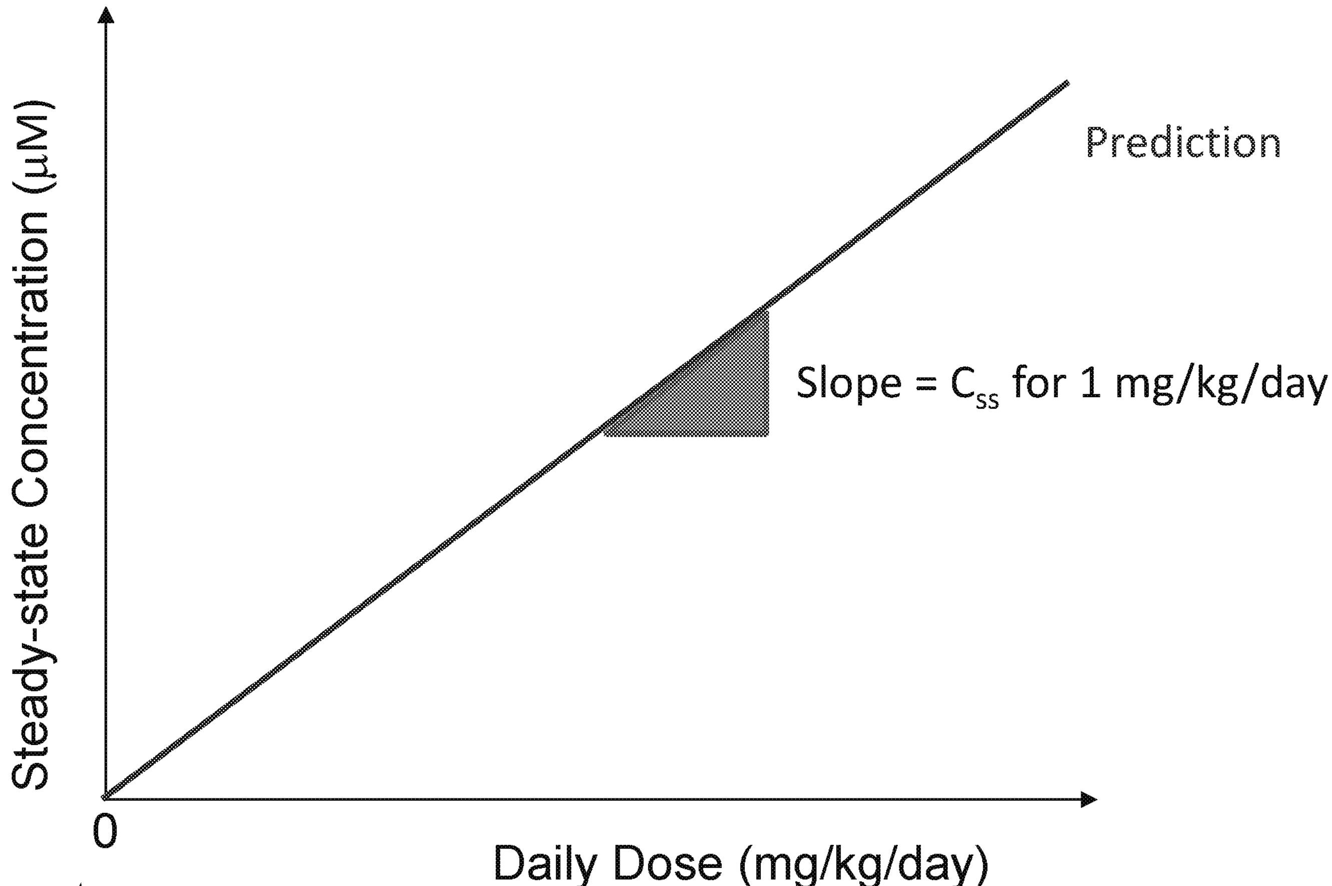
Steady-State is Linear with Dose



$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * F_{ub}) + \left(Q_l * F_{ub} * \frac{Cl_{int}}{Q_l + F_{ub} * Cl_{int}}\right)}$$

- Can calculate predicted steady-state concentration (C_{ss}) for a 1 mg/kg/day dose and multiply to get concentrations for other doses

Wetmore *et al*. (2012)

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Steady-State is Linear with Dose

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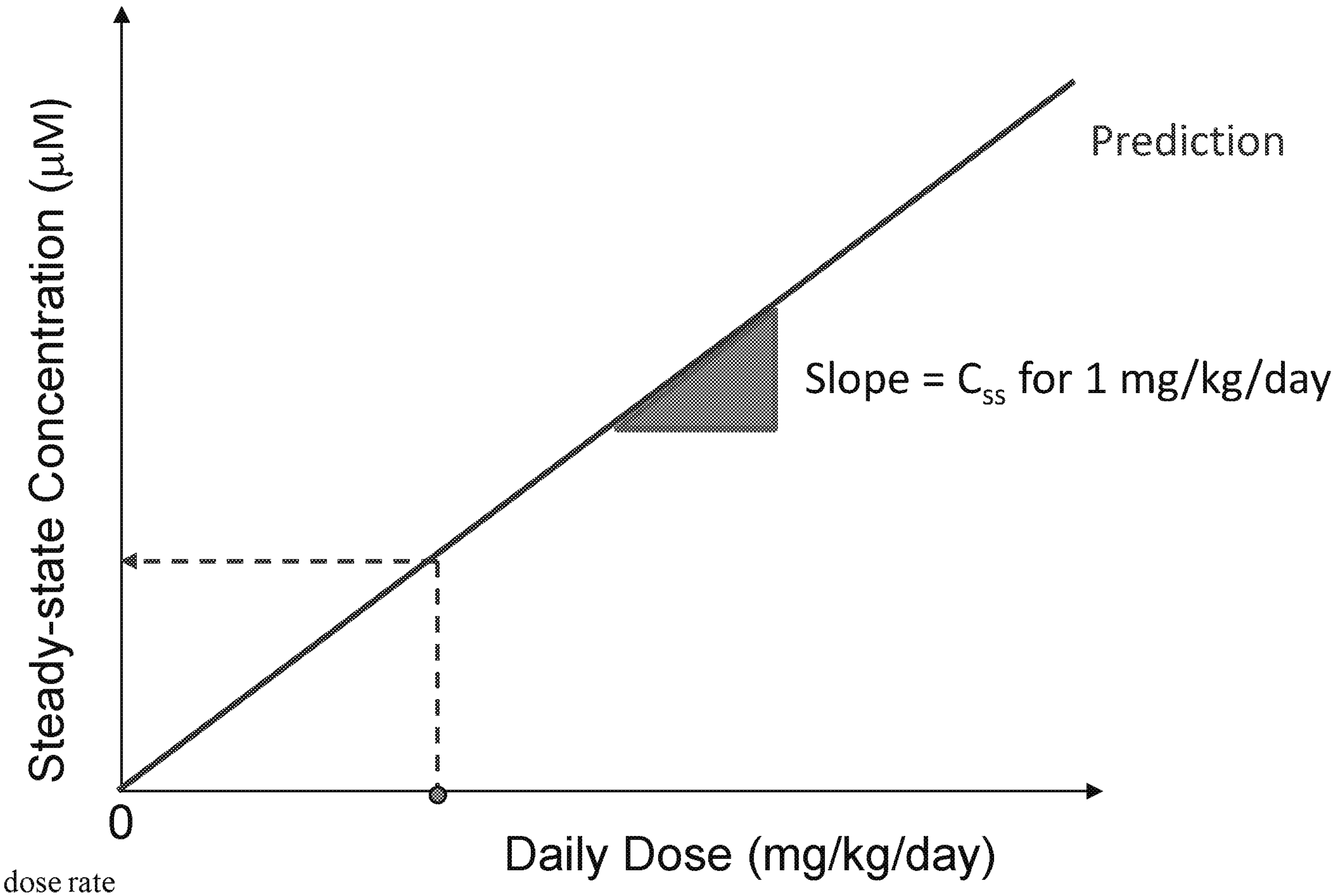
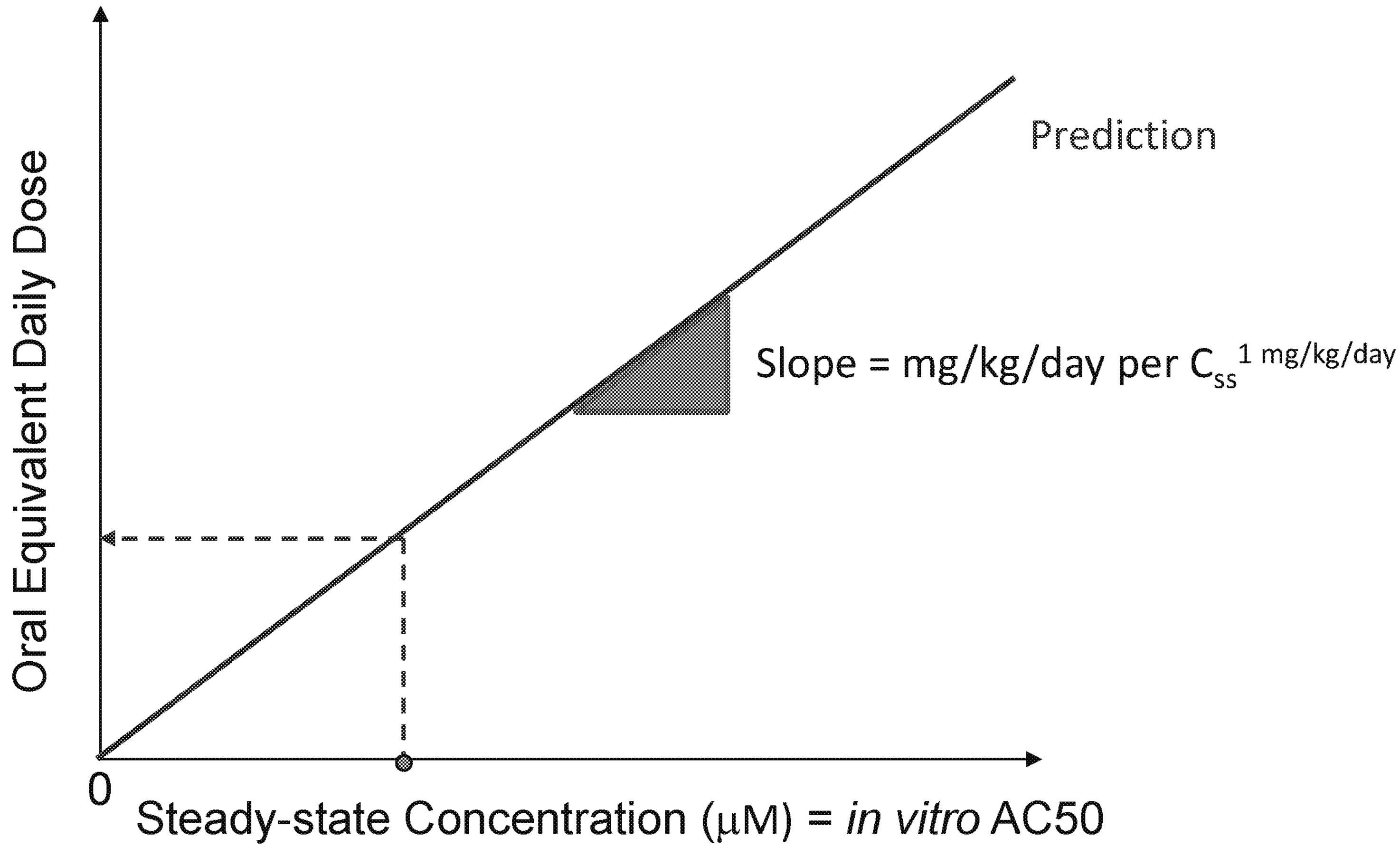


$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{\text{ub}}) + \left(\text{Q}_l * \text{F}_{\text{ub}} * \frac{Cl_{\text{int}}}{\text{Q}_l + \text{F}_{\text{ub}} * Cl_{\text{int}}}\right)}$$

- Can calculate predicted steady-state concentration (C_{ss}) for a 1 mg/kg/day dose and multiply to get concentrations for other doses

Wetmore *et al.* (2012)

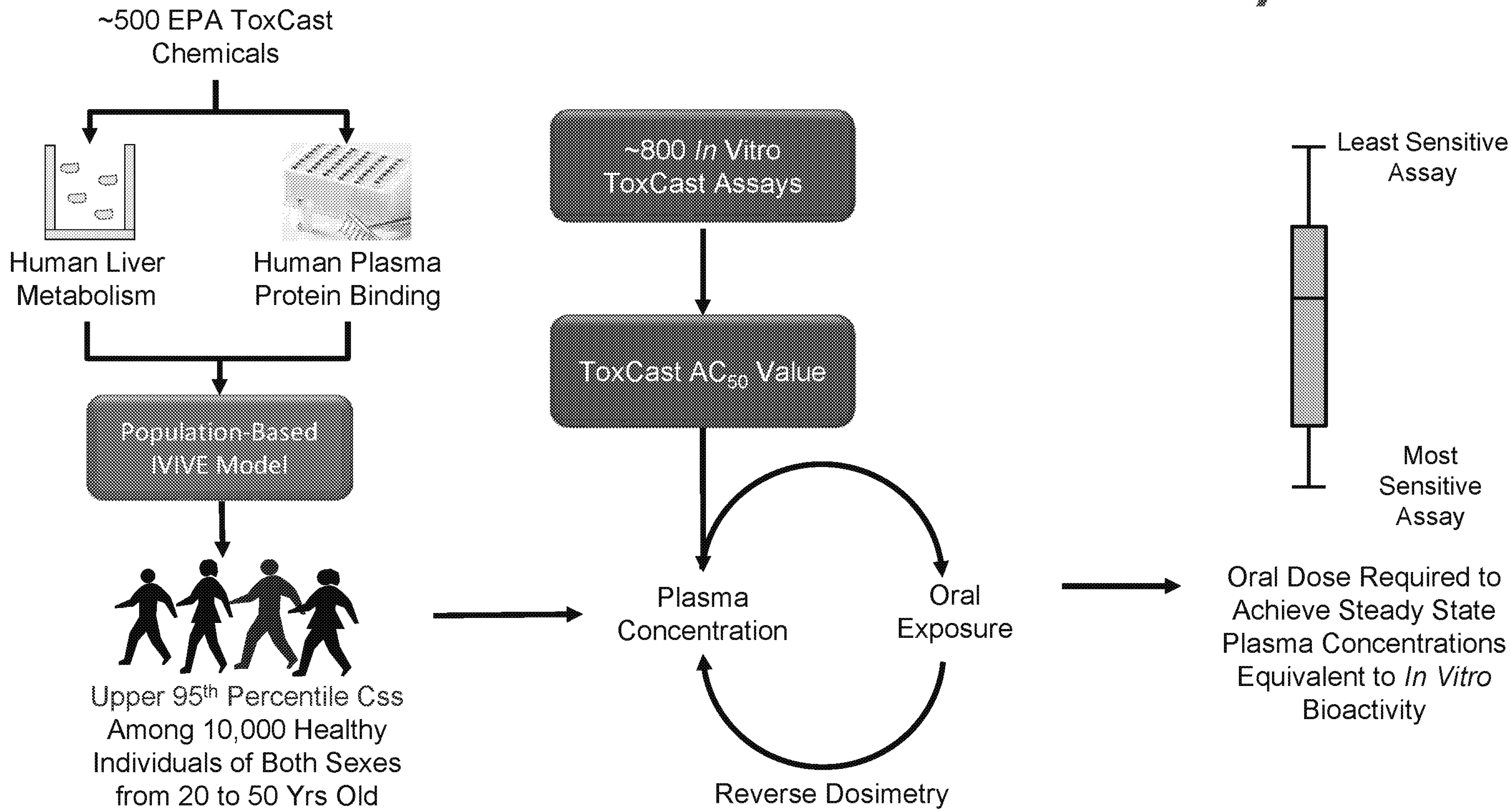
HTTK Allows Steady-State *In Vitro-In Vivo* Extrapolation (IVIVE)



- Swap the axes (this is the "reverse" part of reverse dosimetry)
- Can divide bioactive concentration by C_{ss} for for a 1 mg/kg/day dose to get oral equivalent dose

Wetmore *et al*. (2012)

Integrating Human Dosimetry and Exposure with ToxCast *In Vitro* Assays



Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.*, 2012
Wetmore et al., *Tox Sci*, 2015

Slide from Barbara Wetmore

ToxCast *in vitro* Bioactive Concentrations

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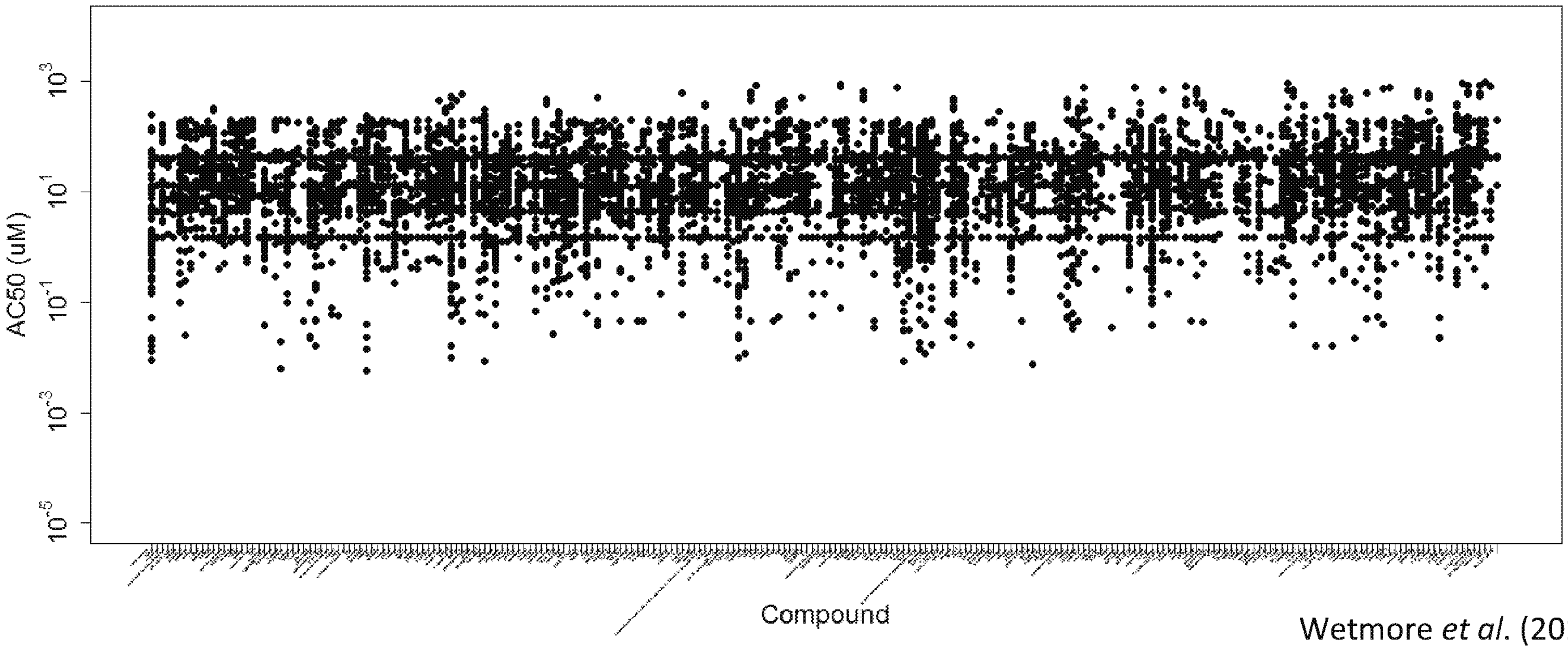
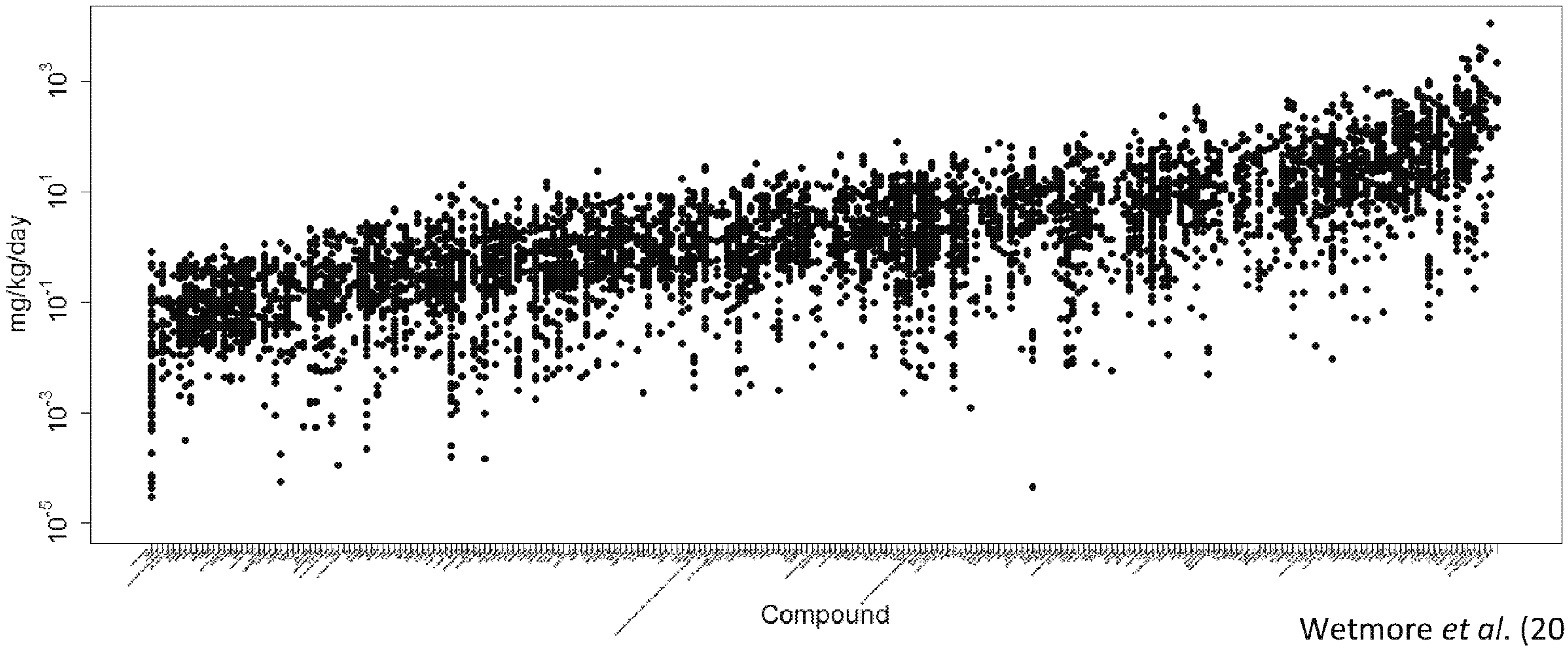


Wetmore *et al.* (2012)

- It appears harder to prioritize on bioactive *in vitro* concentration without *in vivo* context

HTTK Oral Equivalents



Wetmore *et al*. (2012)

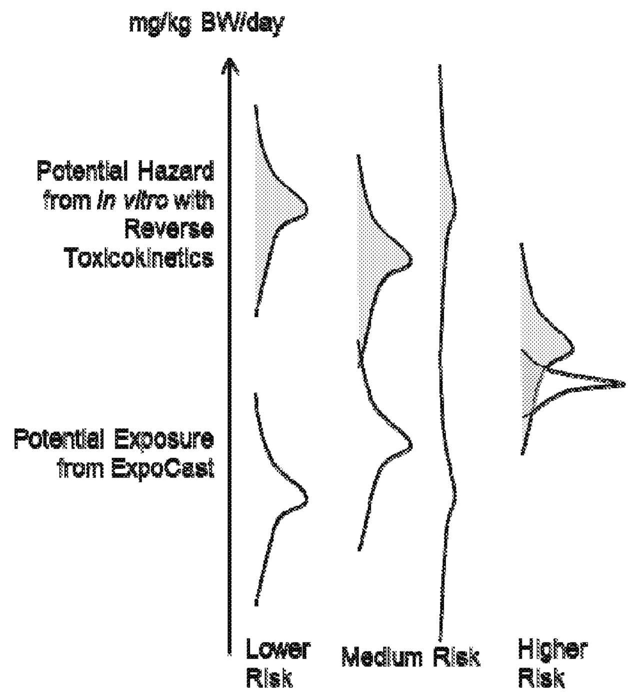
- Translation from *in vitro* to steady-state oral equivalent doses allow greater discrimination between effective chemical potencies

Activity-Exposure Ratio
(Wetmore et al. 2012, 2014, 2015)

$$\text{AER} = \frac{\text{Oral Equiv. Dose}}{\text{Estimated exposure}}$$

AER <=1 : Exposure potentially high enough to cause bioactivity

AER >> 1: Exposure less likely to be high enough to cause bioactivity



Slide from Caroline Ring

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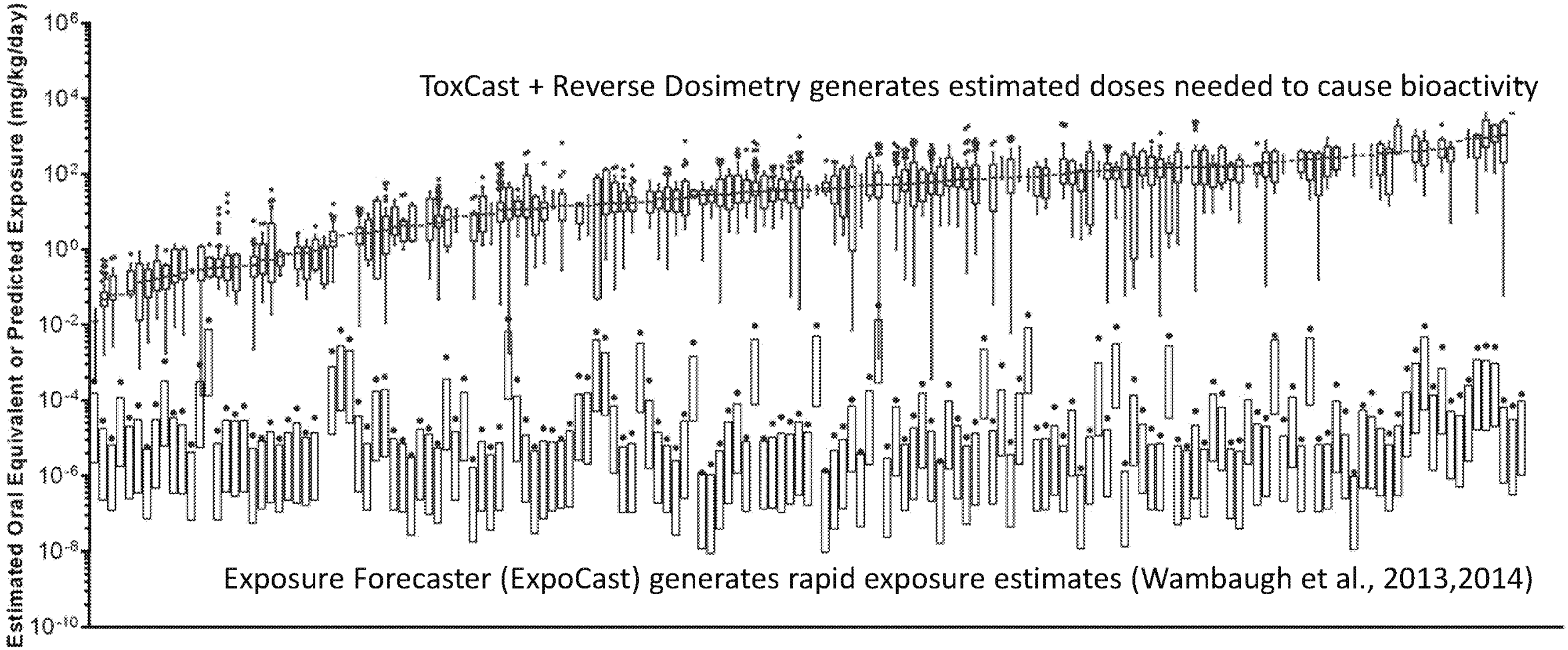
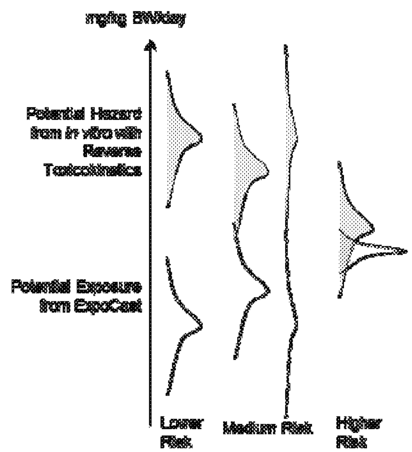
Incorporating Dosimetry-Adjusted ToxCast Bioactivity Data with Exposure





Wetmore *et al.*, Tox. Sci, 2015

Variability in this Steady-State TK Model

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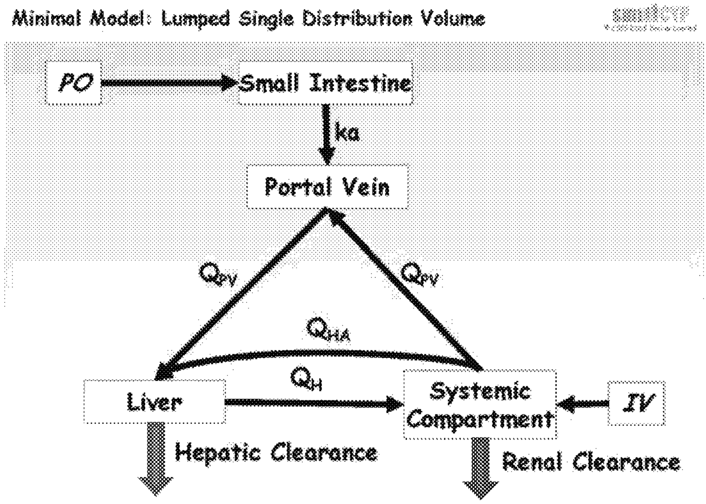
Jamei *et al.* (2009)



$$C_{ss} = \frac{\text{oral dose rate}}{\underbrace{\left(\mathrm{GFR} * \mathrm{F_{up}}\right)}_{\text{(Passive) Renal Clearance}} + \underbrace{\left(\mathrm{Q_l} * \mathrm{F_{up}} * \frac{Cl_{\mathrm{int}}}{\mathrm{Q_l} + \mathrm{F_{up}} * Cl_{\mathrm{int}}}\right)}_{\text{Hepatic Clearance (Metabolism)}}}$$

- *In vitro* clearance (μL/min/10^6 hepatocytes) is scaled to a whole organ clearance using the density of hepatocytes per gram of liver and the volume of the liver (which varies between individuals)
- Glomerular filtration rate (GFR) and blood flow to the liver (Q_l) both vary from individual to individual
- Further assume that measured HTTK parameters have 30% coefficient of variation

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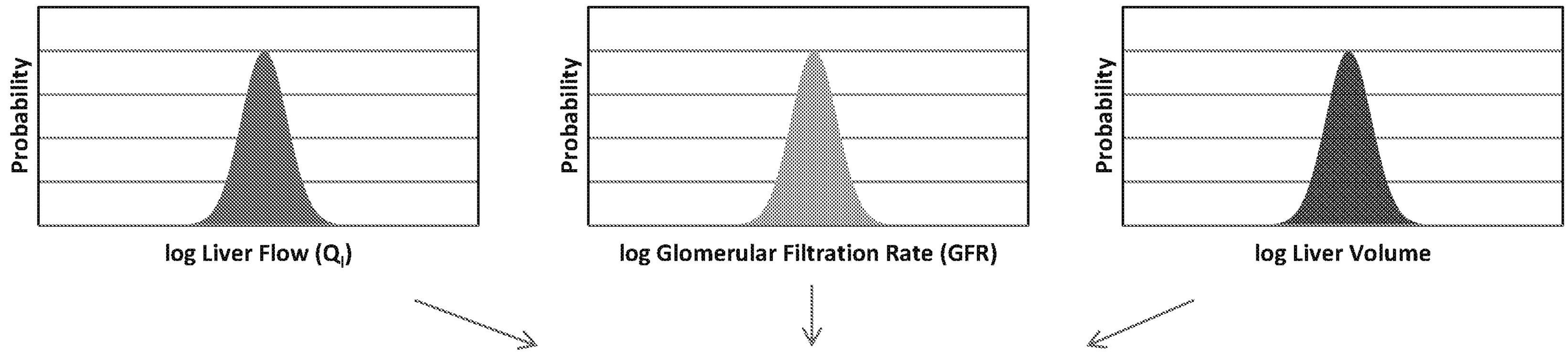
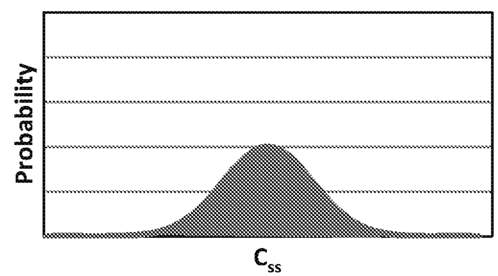
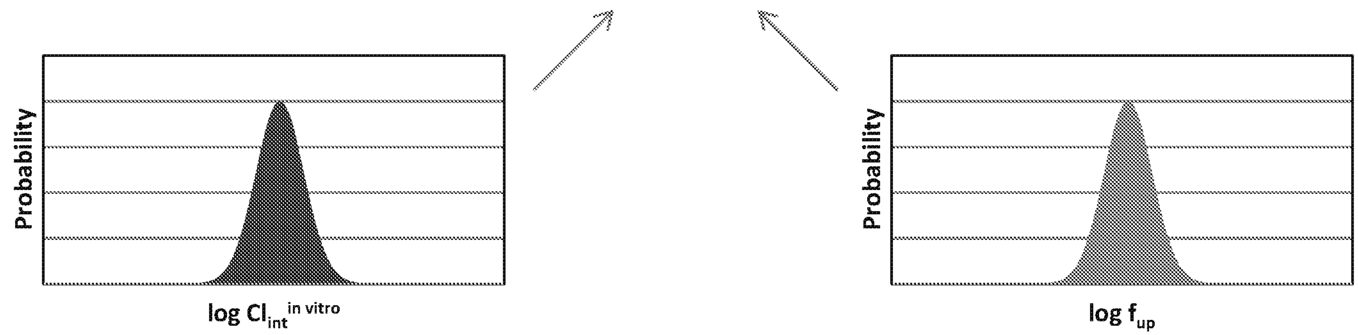
Monte Carlo (MC) Approach to Variability



$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{\text{up}}) + \left(\text{Q}_{\text{l}} * \text{F}_{\text{up}} * \frac{Cl_{\text{int}}}{\text{Q}_{\text{l}} + \text{F}_{\text{up}} * Cl_{\text{int}}}\right)}$$





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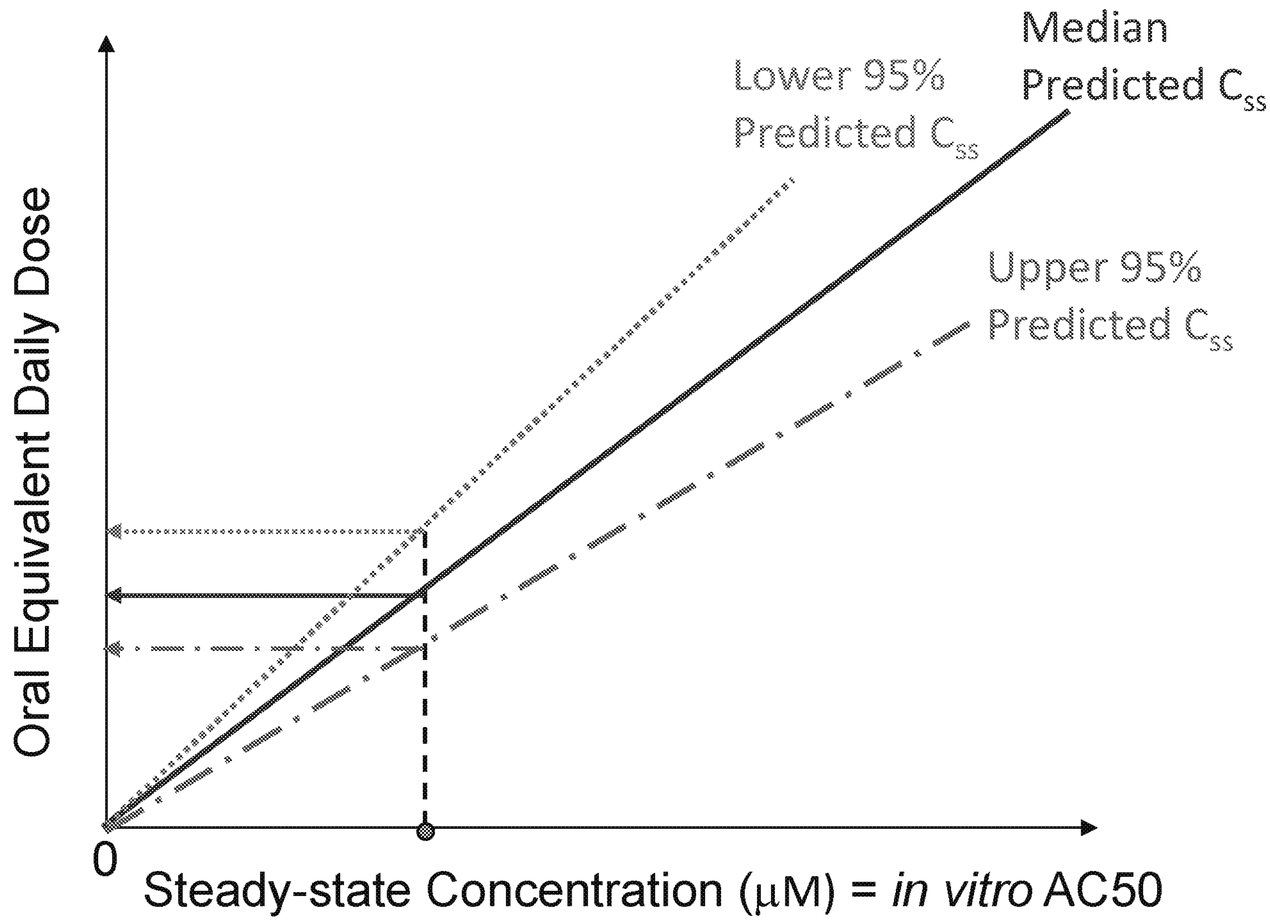
Steady-State In Vitro-In Vivo Extrapolation (IVIVE)



- The higher the predicted C_{ss}, the lower the oral equivalent dose, so the upper 95% predicted C_{ss} from the MC has a lower oral equivalent dose

HTTK Limitations (from Ring et al., 2017)

- Oral absorption
  - 100% assumed, but may be very different
  - *In silico* models not necessarily appropriate for environmental chemicals
- Hepatic Clearance (CL_{int})
  - Ten donor pool in suspension for 2-4 h misses variability and low turnover compounds
  - Isozyme abundances and activity: varies with age, ethnicity (at least) (Yasuda et al. 2008, Howgate et al. 2006, Johnson et al. 2006)
  - Parent chemical depletion only
- Isozyme-specific data & modeling (Wetmore et al. 2014)
  - Isozyme-specific metabolism assays not HT
  - *In silico* predictions of isozyme-specific metabolism? Not easy!
    - Existing data is mostly for pharmaceuticals
- Plasma binding assay (F_{up})
  - Assay often fails due to analytical chemistry sensitivity (Wetmore et al., 2012)
  - Plasma protein concentration variability (Johnson et al. 2006, Israili et al. 2001)
  - Albumin or AAG binding? (Routledge 1986)

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Plasma Protein Binding Assay is Limited by Analytical Chemistry

Rapid Equilibrium Dialysis (RED) Method: Waters *et al.* (2008)

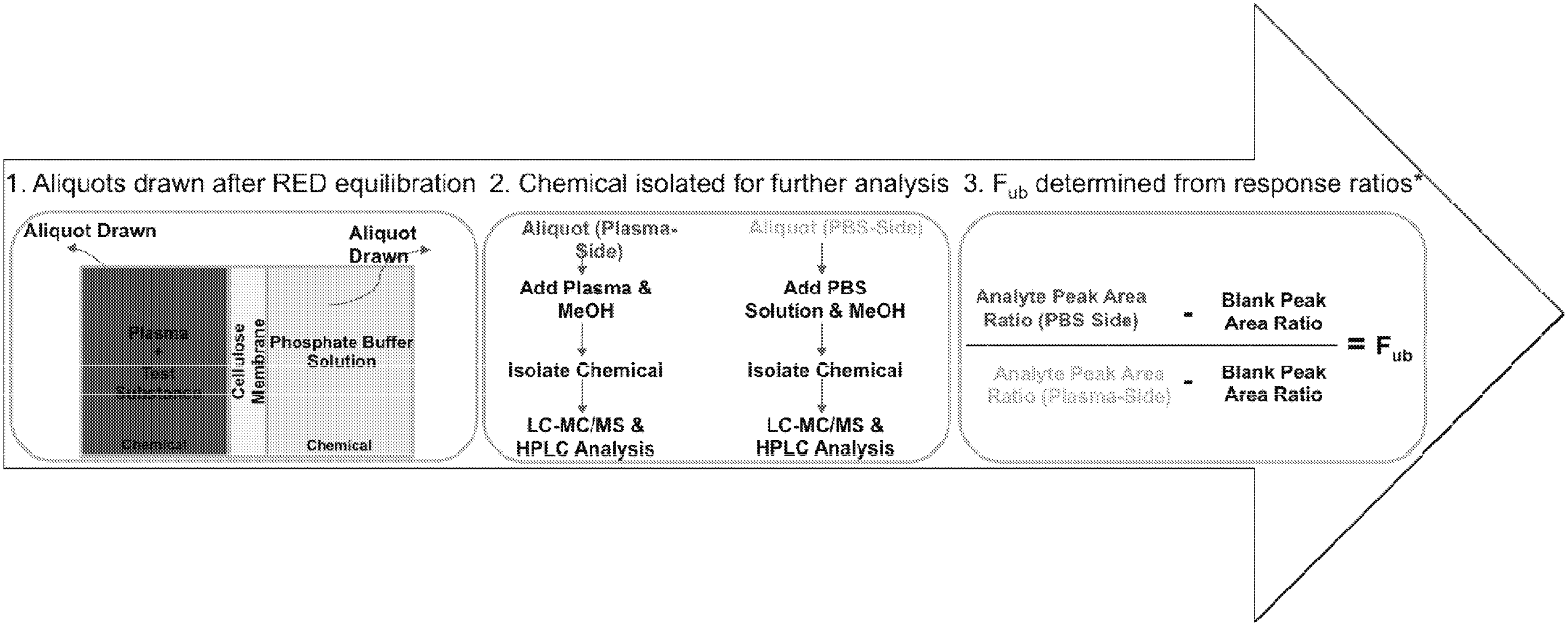

Figure from Chantel Nicolas

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Why Build Another PBTK Tool?

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	SimCYP	ADMET Predictor / GastroPlus	MEGen	httk
Maker	SimCYP Consortium / Certara	Simulations Plus	UK Health and Safety Laboratory (Loizou)	US EPA
Availability	License, but inexpensive for research	License, but inexpensive for research	Free: http://xnet.hsl.gov.uk/megen	Free: CRAN Repository
Population Variability Monte Carlo	Yes	No	No	Yes
Batch Mode	Yes	Yes	No	Yes
Physiological Data	Yes	Yes	Yes	Yes
Chemical-Specific Data Library	Clinical Drugs	No	No	Pharma and ToxCast Compounds: 443 PBTK, +100 steady-state only
Export Function	No	No	Matlab and AcslX	SBML and Jarnac
R Integration	No	No	No	Yes
Easy Reverse Dosimetry	Yes	Yes	No	Yes
Future Proof XML	No	No	Yes	No

We want to do a statistical analysis (using R) for as many chemicals as possible

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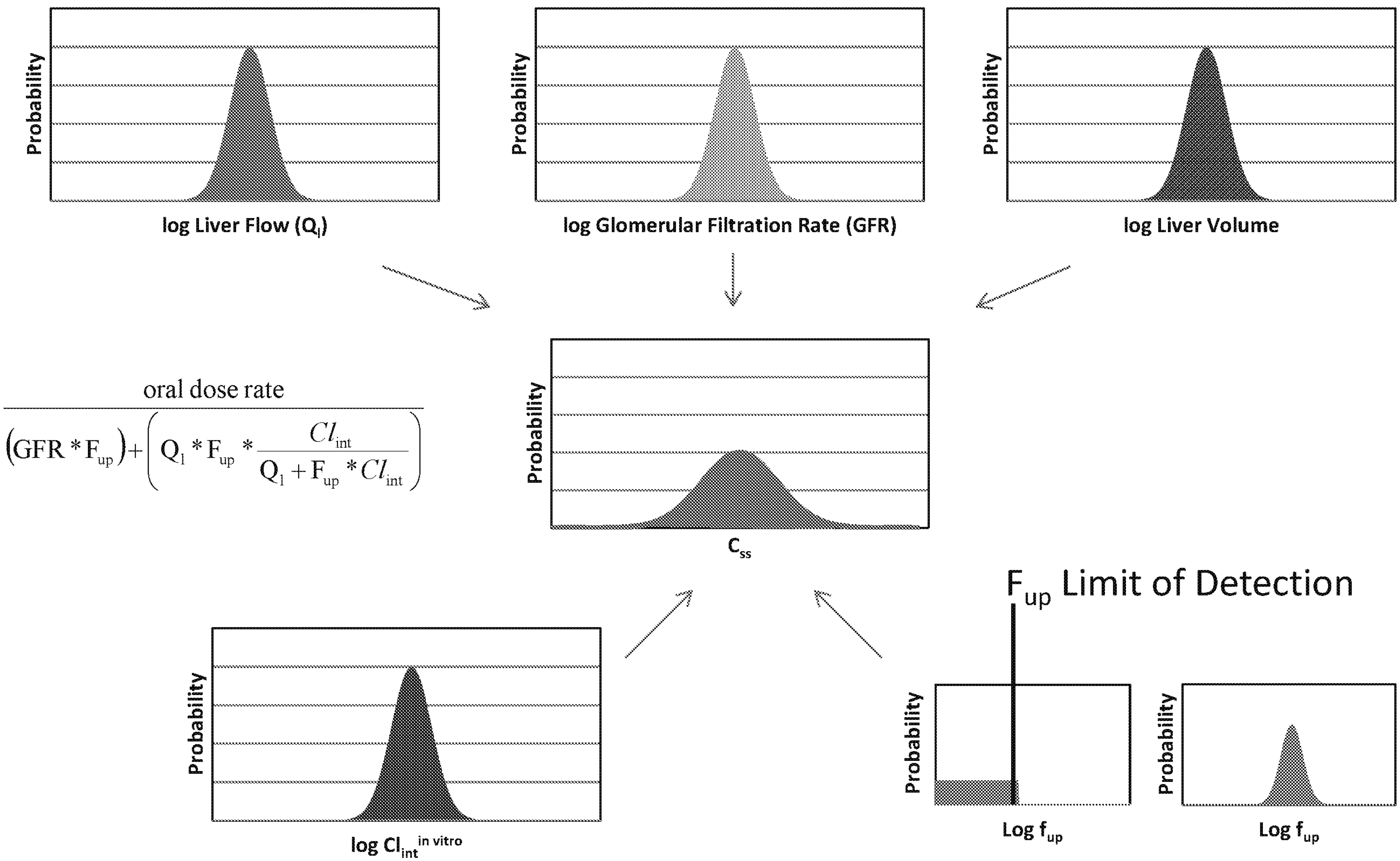
Modified f_{up} Distribution for HTTK



$$C_{ss} = \frac{\text{oral dose rate}}{\left(\text{GFR} * \text{F}_{\text{up}}\right) + \left(\text{Q}_{\text{l}} * \text{F}_{\text{up}} * \frac{Cl_{\text{int}}}{\text{Q}_{\text{l}} + \text{F}_{\text{up}} * Cl_{\text{int}}}\right)}$$

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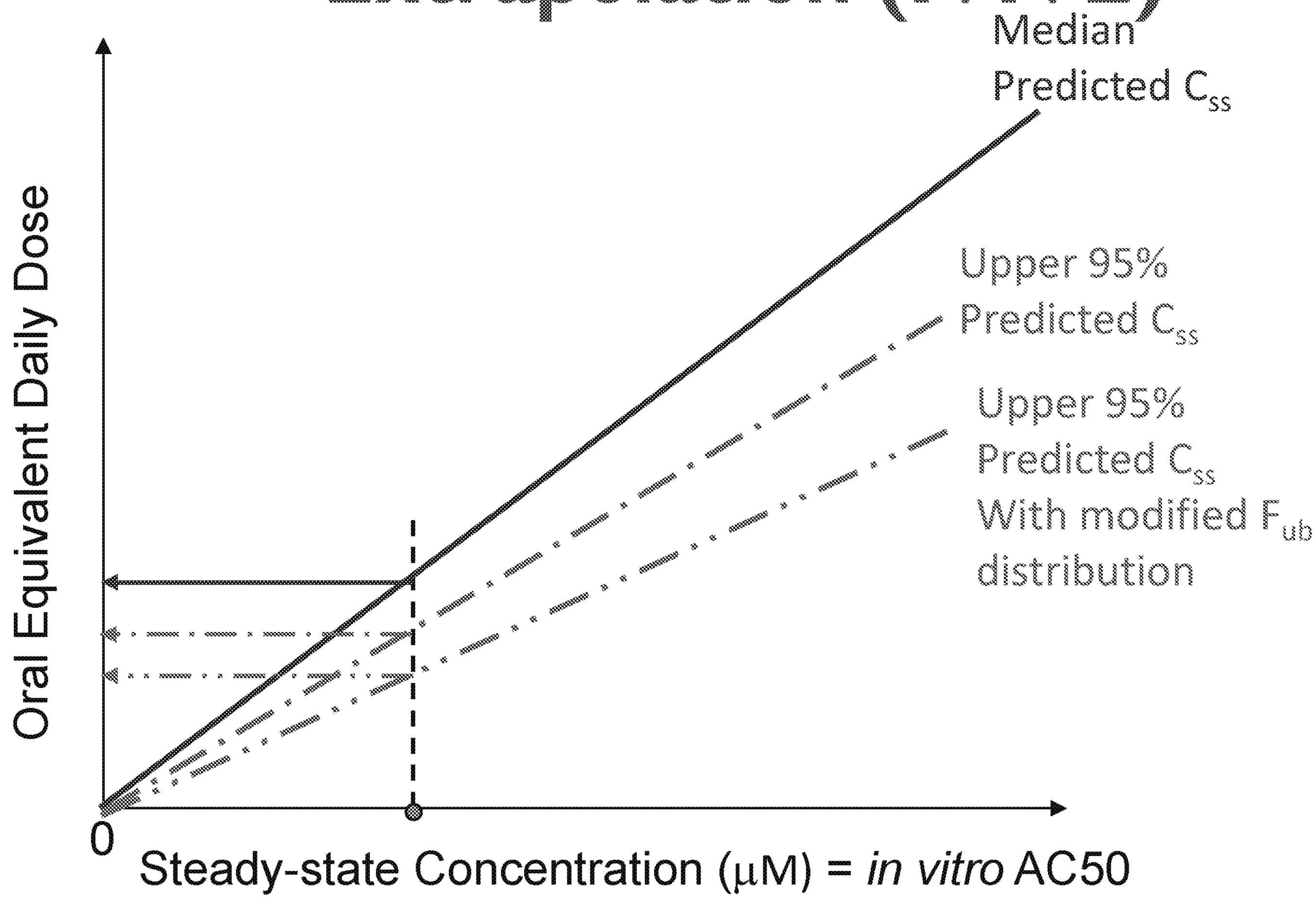
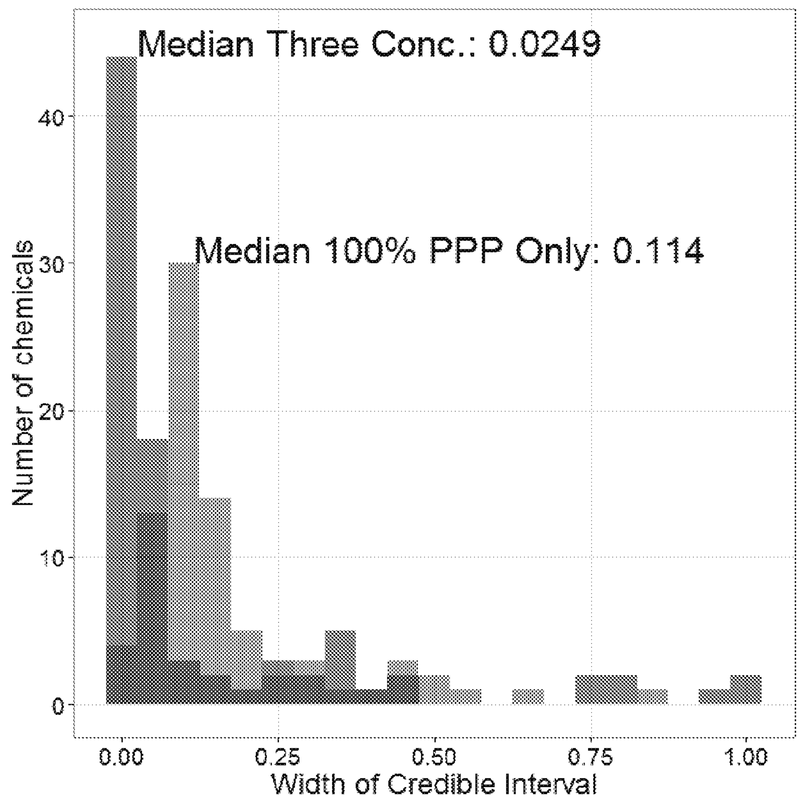
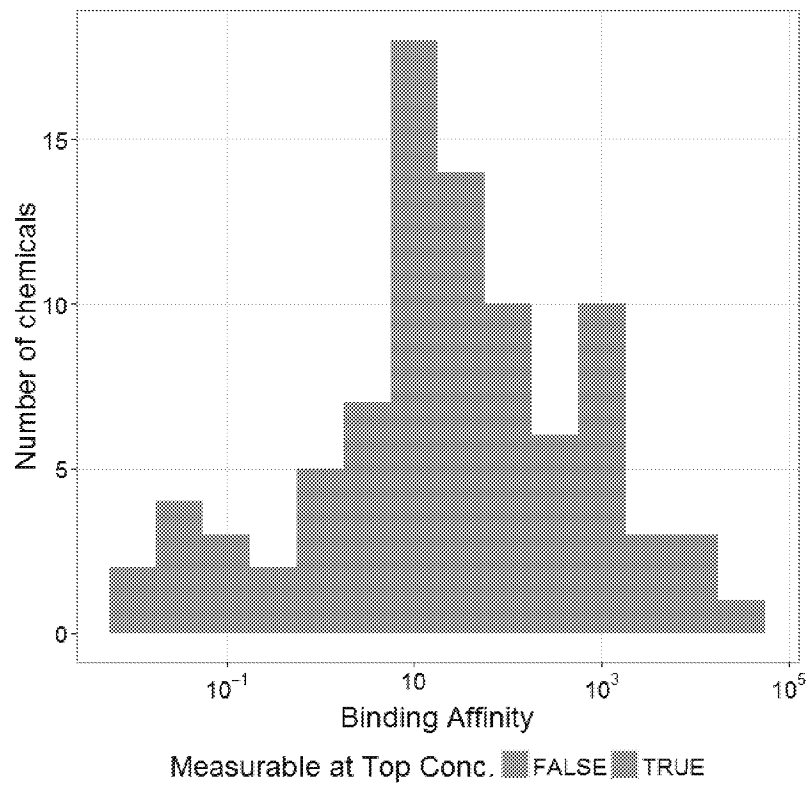
Steady-State In Vitro-In Vivo Extrapolation (IVIVE)



- Taking into account the limit of detection issues does not change the median (or lower 95% C_{ss}) but does change the upper C_{ss}, causing lower oral equivalent dose predictions (greater sensitivity)

Improving Plasma Binding Measurement





- Using a Bayesian analysis via MCMC (in JAGS) to estimate 95% credible intervals
- New protocol uses three plasma protein concentrations (100%, 30%, and 10% of physiologic concentration)
- Can analyze data jointly using a binding affinity model

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Goals for HTTK

- In order to address greater numbers of chemicals we collect *in vitro*, high throughput toxicokinetic (HTTK) data
- The goal of HTTK is to provide a human dose context for in vitro concentrations from HTS
    - This allows direct comparisons with exposure
- An R statistical package allows us to evaluate *in vitro* predictions two ways:
    - We compare *in vitro* predictions and *in vivo* measurements
    - We perform simulation studies to examine key assumptions

R Package "httk"

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CRAN - Package httk

https://cran.r-project.org/web/packages/httk/index.html

Apps DSStox Confluence JESEE EHP Battelle Box

httk: High-Throughput Toxicokinetics

Functions and data tables for simulation and statistical analysis of chemical toxicokinetics ("TK") using data obtained from relatively high throughput, in vitro studies. Both physiologically-based ("PBTK") and empirical (e.g., one compartment) "TK" models can be parameterized for several hundred chemicals and multiple species. These models are solved efficiently, often using compiled (C-based) code. A Monte Carlo sampler is included for simulating biological variability and measurement limitations. Functions are also provided for exporting "PBTK" models to "SBML" and "JARNAC" for use with other simulation software. These functions and data provide a set of tools for in vitro-in vivo extrapolation ("IVIVE") of high throughput screening data (e.g., ToxCast) to real-world exposures via reverse dosimetry (also known as "RTK").

Version:	1.6
Depends:	R (≥ 2.10)
Imports:	deSolve, msm, data.table, survey, mvtnorm, truncnorm, stats, utils
Suggests:	ggplot2, knitr, rmarkdown, R.rsp, GGally, gplots, scales, EnvStats, MASS, RColorBrewer, TeachingDemos, classInt, ks, reshape2, gdata, viridis, CensRegMod, gmodels, colorspace
Published:	2017-06-08
Author:	John Wambaugh, Robert Pearce, Caroline Ring, Jimena Davis, Nisha Sipes, and R. Woodrow Setzer
Maintainer:	John Wambaugh <wambaugh.john at epa.gov>
License:	GPL-3
NeedsCompilation:	yes
Materials:	NEWS
CRAN checks:	httk results

Downloads:

Reference manual:	httk.pdf
Vignettes:	Creating Partition Coefficient Evaluation Plots Age distributions Global sensitivity analysis Global sensitivity analysis plotting Height and weight spline fits and residuals Hematocrit spline fits and residuals Plotting Css95 Serum creatinine spline fits and residuals Generating subpopulations Evaluating HTTK models for subpopulations Generating Figure 2 Generating Figure 3 Plotting Howgate/Johnson data AER plotting Virtual study populations httk: R Package for High-Throughput Toxicokinetics

https://CRAN.R-project.org/package=httk

Can access this from the R GUI:

"Packages" then "Install Packages"

- "httk" R Package for reverse dosimetry and PBTK
- 553 chemicals to date
- 100's of additional chemicals being studied
- Pearce *et al.* documentation manuscript accepted at Journal of Statistical Software
- Vignettes provide examples of how to use many functions

Within R: type "help(httk)"

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127.0.0.1:24930/library/httk/html/httk-package.html

httk-package {httk} R Documentation

High-Throughput Toxicokinetics httk: High-Throughput Toxicokinetics

Description

Functions and data tables for simulation and statistical analysis of chemical toxicokinetics ("TK") using data obtained from relatively high throughput, in vitro studies. Both physiologically-based ("PBTK") and empirical (e.g., one compartment) "TK" models can be parameterized for several hundred chemicals and multiple species. These models are solved efficiently, often using compiled (C-based) code. A Monte Carlo sampler is included for simulating biological variability and measurement limitations. Functions are also provided for exporting "PBTK" models to "SBML" and "JARNAC" for use with other simulation software. These functions and data provide a set of tools for in vitro-in vivo extrapolation ("IVIVE") of high throughput screening data (e.g., ToxCast) to real-world exposures via reverse dosimetry (also known as "RTK"). Functions and data tables for simulation and statistical analysis of chemical toxicokinetics ("TK") using data obtained from relatively high throughput, in vitro studies. Both physiologically-based ("PBTK") and empirical (e.g., one compartment) "TK" models can be parameterized for several hundred chemicals and multiple species. These models are solved efficiently, often using compiled (C-based) code. A Monte Carlo sampler is included for simulating biological variability and measurement limitations. Functions are also provided for exporting "PBTK" models to "SBML" and "JARNAC" for use with other simulation software. These functions and data provide a set of tools for in vitro-in vivo extrapolation ("IVIVE") of high throughput screening data (e.g., ToxCast) to real-world exposures via reverse dosimetry (also known as "RTK").

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Caroline Ring

Nisha Sipes

Jimena Davis

R. Woodrow Setzer

See Also

Useful links:

https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=311211

https://www.epa.gov/chemical-research/rapid-chemical-exposure-and-dose-research

https://doi.org/10.1093/toxsci/kfv171

https://doi.org/10.1093/toxsci/kfv118

[Package *httk* version 1.6 Index]

Within R: type "help(httk)"

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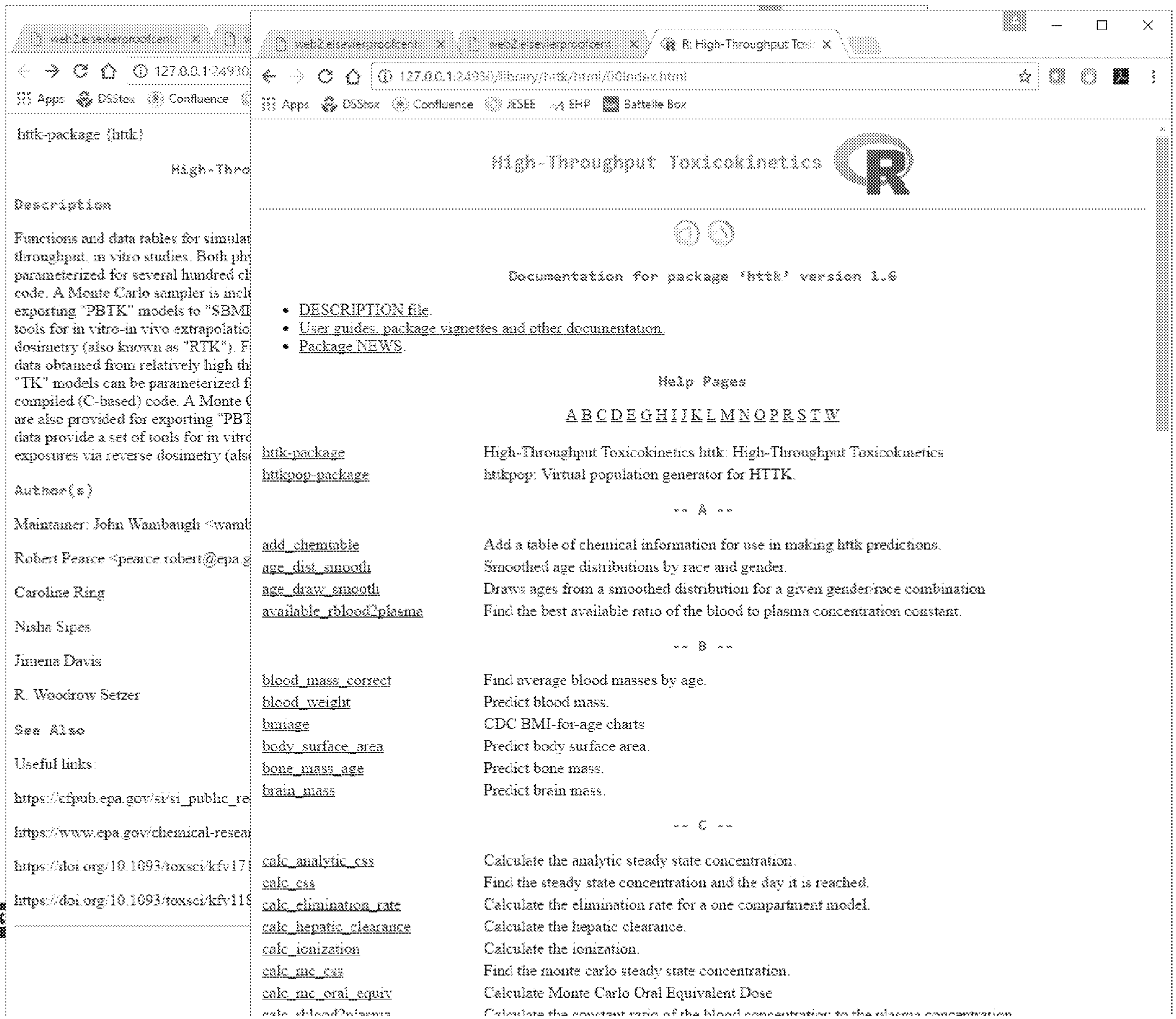

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Within R: type "help(httk)"

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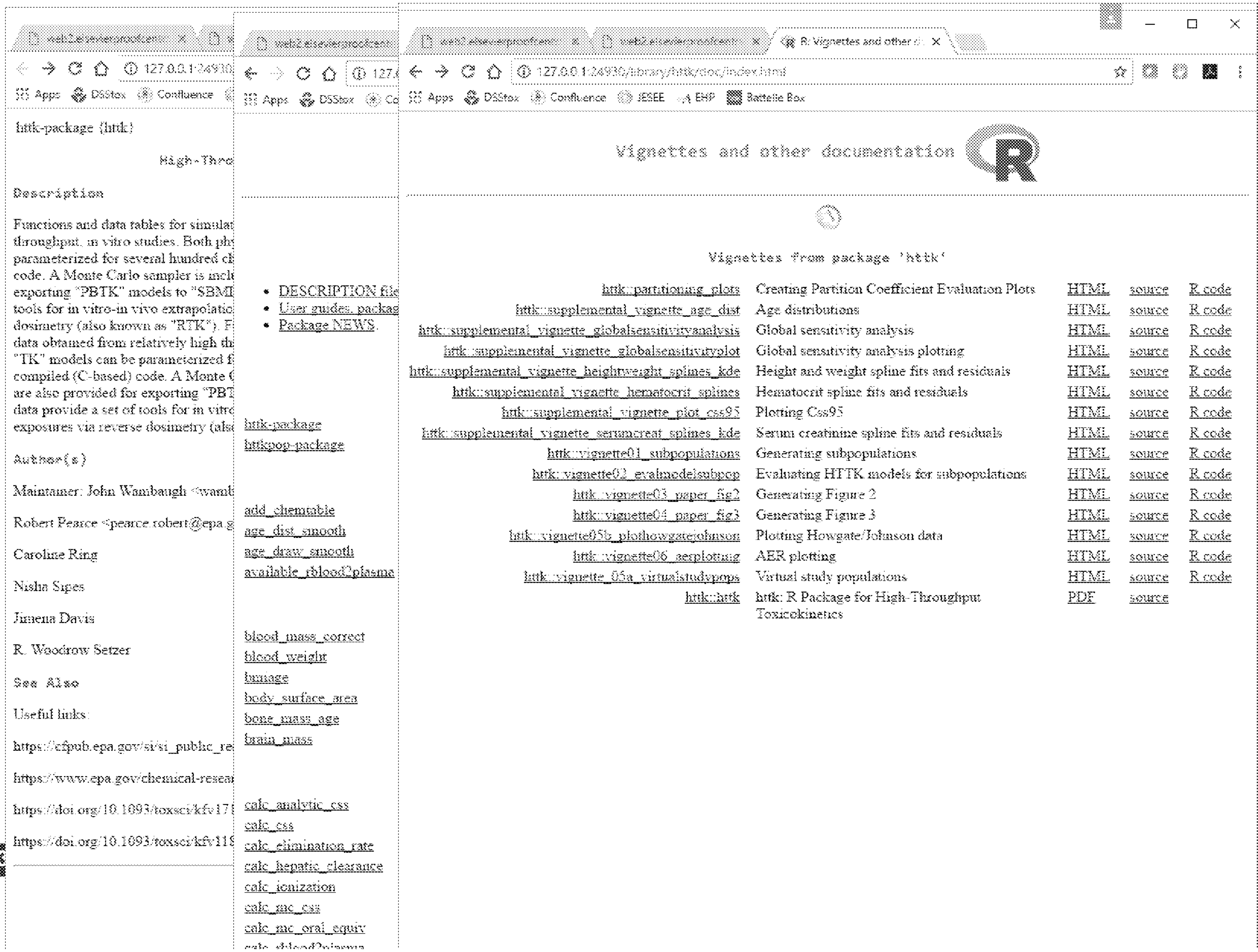

httk::partitioning_plots	Creating Partition Coefficient Evaluation Plots	HTML	source	R code
httk::supplemental_vignette_age_dist	Age distributions	HTML	source	R code
httk::supplemental_vignette_globalsensitivityanalysis	Global sensitivity analysis	HTML	source	R code
httk::supplemental_vignette_globalsensitivityplot	Global sensitivity analysis plotting	HTML	source	R code
httk::supplemental_vignette_heightweight_splines_kde	Height and weight spline fits and residuals	HTML	source	R code
httk::supplemental_vignette_hematocrit_splines	Hematocrit spline fits and residuals	HTML	source	R code
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httk::vignette_05a_virtualstudypops	Virtual study populations	HTML	source	R code
httk::httk	httk: R Package for High-Throughput Toxicokinetics	PDF	source	

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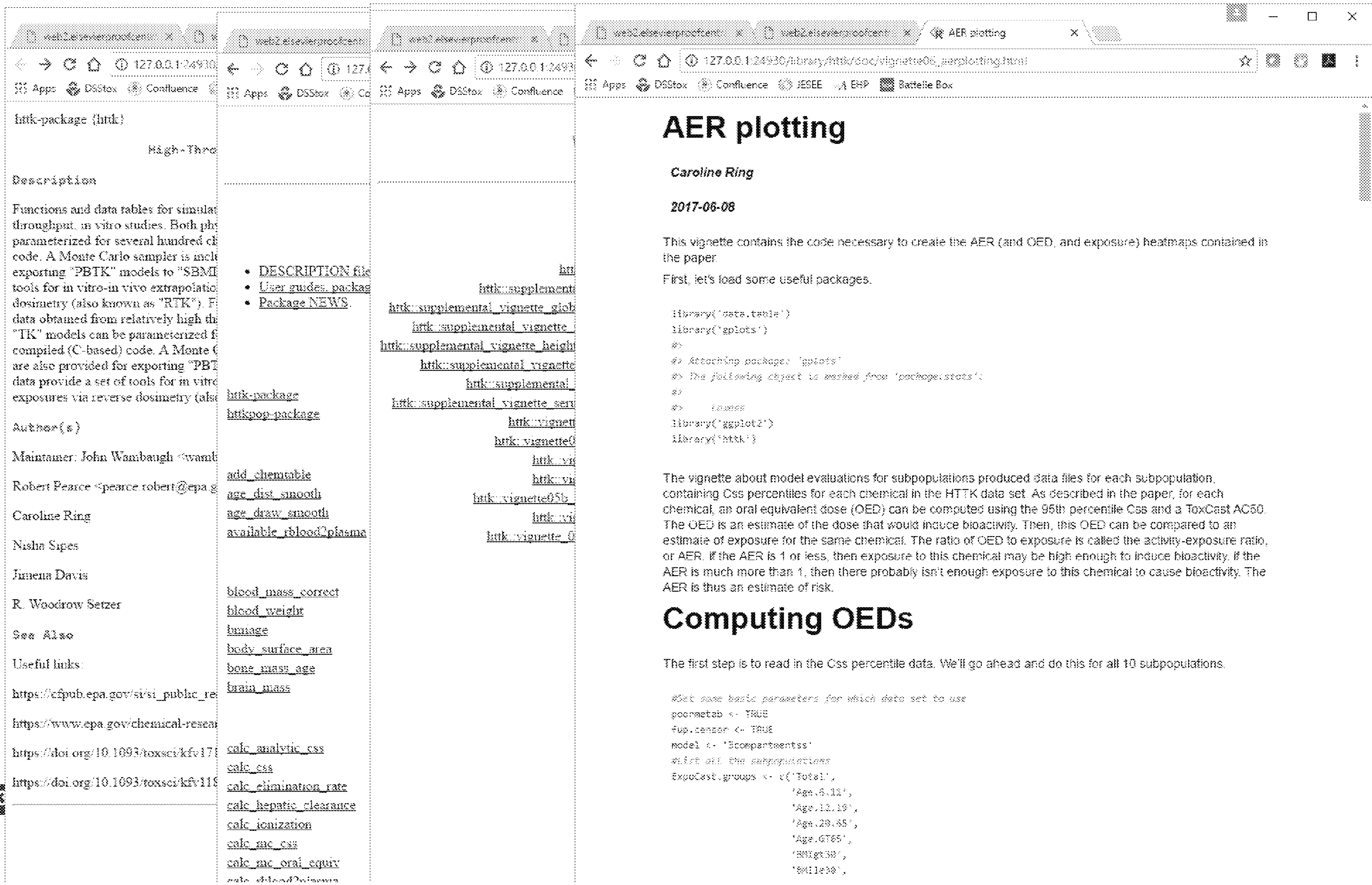
Within R: type "help(httk)"



EPA United States Environmental Protection Agency

What you can do with R Package "httk"

- Allows, one compartment, two-compartment, three-compartment, and PBTK modeling
- Allows conversion of *in vitro* concentration to *in vivo* doses
- Allows prediction of internal tissue concentrations from dose regimen (oral and intravenous)
- A peer-reviewed paper in the Journal of Statistical software provides a how-to guide (Pearce et al., *in press*)
- You can use the built in chemical library or add more chemical information (examples provided in JSS paper)
- You can load specific (older) versions of the package
- You can use specific demographics in the population simulator (v1.5 and later – Ring et al., *in press*)
    - Gender, age, weight, ethnicity, renal function
- You can control the built in random number generator to reproduce the same random sequence

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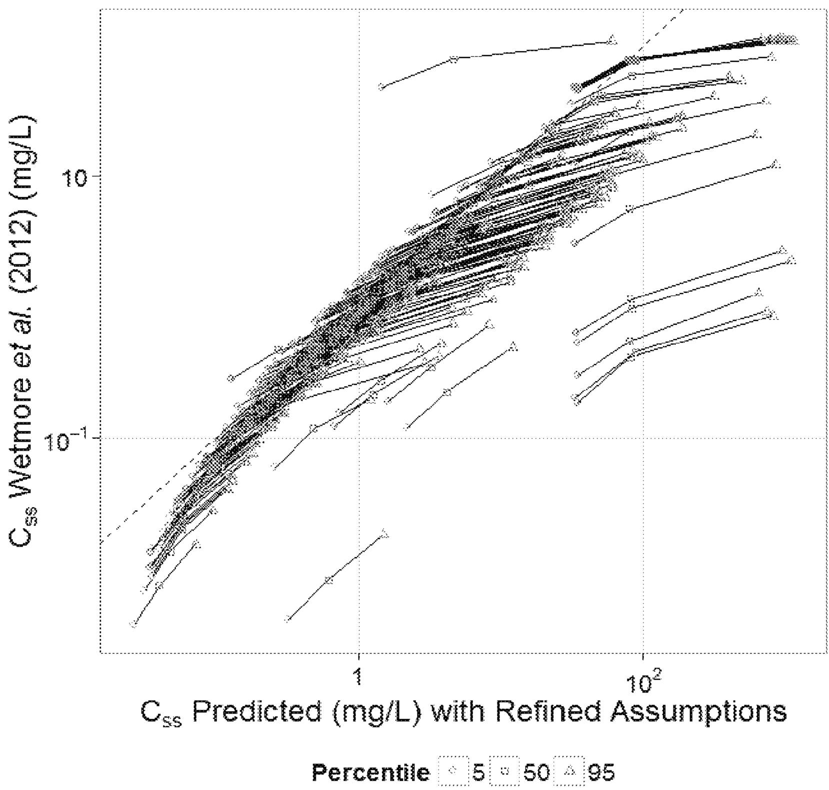
Comparison Between httk and SimCYP



- In the Rotroff *et al*. (2010) and Wetmore et al. (2012,2013,2014,2015) papers SimCYP was used to predict distributions of C_{ss} from *in vitro* data
  - We show that "httk" can reproduce the results from those publications for most chemicals using our implementation of Monte Carlo.
- Any one chemical's median and quantiles are connected by a dotted line.

- The RED assay for measuring protein binding fails in some cases because the amount of free chemical is below the limit of detection
  - A default value of 0.5% free was used
  - Now we use random draws from a uniform distribution from 0 to 1%.

Wambaugh et al. (2015)

United States Environmental Protection Agency

Steady State Concentration Examples

```
library(httk)

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for human for Acetochlor (published value):
calc_mc_css(chem.cas="34256-82-1“)

# Should produce error:
calc_mc_css(chem.name="34256-82-1")

#Capitalization shouldn’t matter:
calc_mc_css(chem.name="acetochlor"
calc_mc_css(chem.name="Acetochlor“)

# What’s going on?
help(calc_mc_css)

# What chemicals can I do?
get_cheminfo()
```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Oral Equivalent Dose Examples

#State-state oral equivalent dose (mg/kg BW/day) to produce 0.1 uM serum concentration for human, 0.95 quantile, for Acetochlor (published value):

```
get_wetmore_oral_equiv(0.1,chem.cas="34256-82-1")
```

#State-state oral equivalent dose (mg/kg BW/day) to produce 0.1 uM serum concentration for human, 0.95 quantile, for Acetochlor (calculated value):

```
calc_mc_oral_equiv(0.1,chem.cas="34256-82-1”)
```

#State-state oral equivalent dose (mg/kg BW/day) to produce 0.1 uM serum concentration for human, 0.05, 0.5, and 0.95 quantile, for Acetochlor (published values):

```
get_wetmore_oral_equiv(0.1,chem.cas="34256-82-1",which.quantile=c(0.05,0.5,0.95))
```

#State-state oral equivalent dose (mg/kg BW/day) to produce 0.1 uM serum concentration for human, 0.05, 0.5, and 0.95 quantiles, for Acetochlor (calculated value):

```
calc_mc_oral_equiv(0.1,chem.cas="34256-82-1",which.quantile=c(0.05,0.5,0.95))
```

#State-state oral equivalent dose (mg/kg BW/day) to produce 0.1 uM serum concentration for rat, 0.95 quantile, for Acetochlor (calculated value):

```
calc_mc_oral_equiv(0.1,chem.cas="34256-82-1",species="Rat“)
```

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Interspecies Extrapolation Examples

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for human for Acetochlor (calculated value):

```
calc_mc_css(chem.cas="34256-82-1",method="dr"))
```

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for rat for Acetochlor (should produce errors since there is no published value, 0.5 quantile only):

```
get_wetmore_css(chem.cas="34256-82-1",species="Rat")
```

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for rat for Acetochlor (calculated value):

```
calc_mc_css(chem.cas="34256-82-1",species="Rat")
```

#Steady-state concentration (uM) for 1 mg/kg/day for 0.5 quantile for rat for Acetochlor (published value):

```
get_wetmore_css(chem.cas="34256-82-1",species="Rat",which.quantile=0.5)
```

#Steady-state concentration (uM) for 1 mg/kg/day for 0.5 quantile for rat for Acetochlor (calculated value):

```
calc_mc_css(chem.cas="34256-82-1",species="Rat",which.quantile=0.5)
```

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for mouse for Acetochlor (should produce error since there is no published value, human and rat only):

```
get_wetmore_css(chem.cas="34256-82-1",species="Mouse")
```

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for mouse for Acetochlor (calculated value):

```
calc_mc_css(chem.cas="34256-82-1",species ="Mouse")
```

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Help Files

Every function has a help file

help(add_chemtable)

Add a table of chemical information for use in making httk predictions.

Description

This function adds chemical-specific information to the table chem.physical_and_invitro.data. This table is queried by the model parameterization functions when attempting to parameterize a model, so adding sufficient data to this table allows additional chemicals to be modeled.

Usage

```
add_chemtable(new.table, data.list, current.table=NULL, reference=NULL,species=NULL, overwrite=F)
```

Arguments

new.table	Object of class data.frame containing one row per chemical, with each chemical minimally by described by a CAS number.
data.list	This list identifies which properties are to be read from the table. Each item in the list should point to a column in the table new.table. Valid names in the list are: 'Compound', 'CAS', 'DSSTox.GSID' 'SMILES.desalt', 'Reference', 'Species', 'MW', 'logP', 'pKa_Donor', 'pKa_Accept', 'logMA', 'Clint', 'Clint.pValue', 'Funbound.plasma', 'Fgutabs', 'Rblood2plasma'. Note that Rblood2plasma (Ratio blood to plasma) is currently not used.

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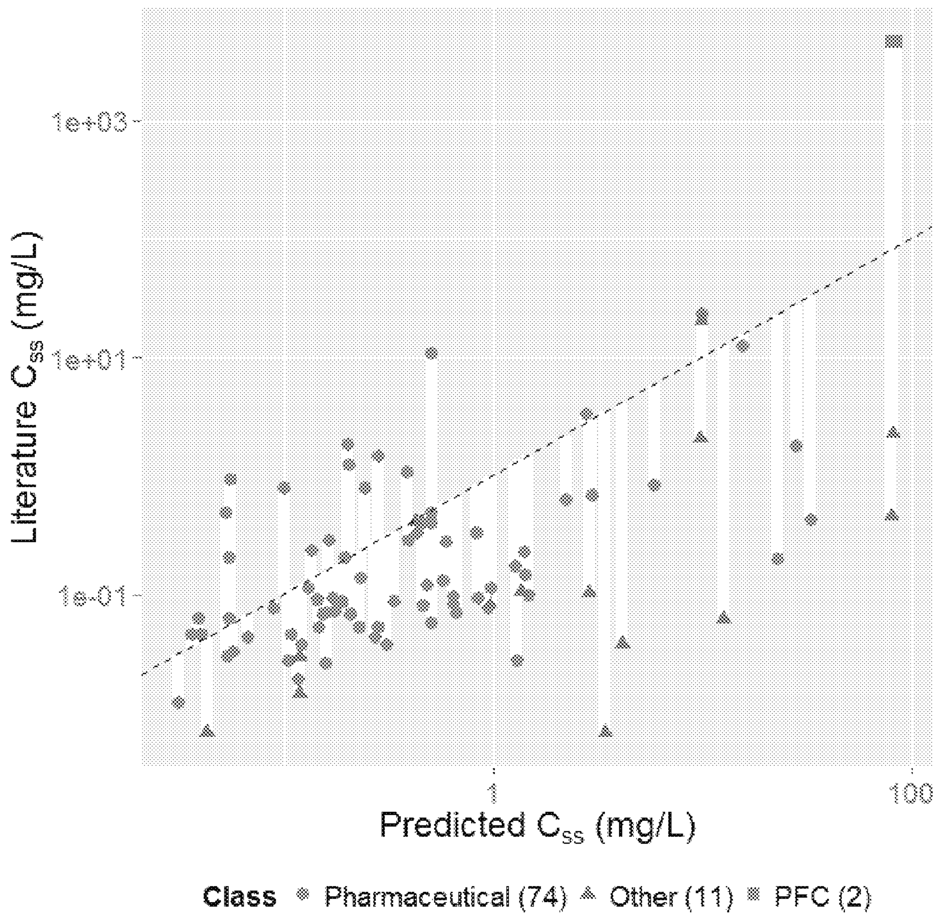
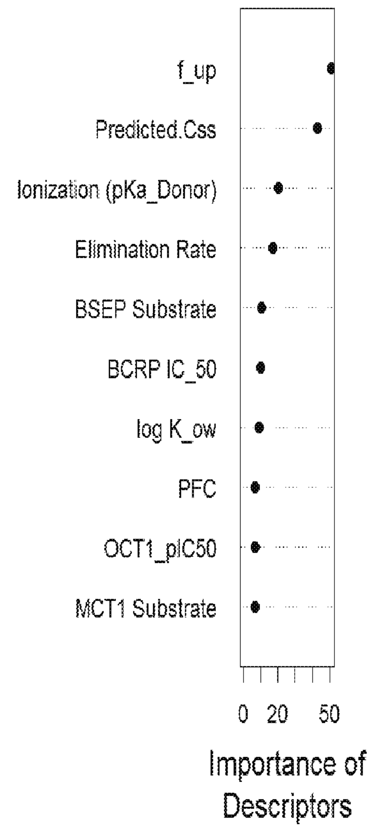
Why Do Statistical Analysis?

- *In vivo* Predictive Ability and Domain of Applicability
- In drug development, HTTK methods estimate therapeutic doses for clinical studies – predicted concentrations are typically on the order of values measured in clinical trials (Wang, 2010)
- For environmental compounds, there will be no clinical trials
- Uncertainty must be well characterized ideally with rigorous statistical methodology
  - We will use direct comparison to *in vivo* data in order to get an empirical estimate of our uncertainty
  - Any approximations, omissions, or mistakes should work to increase the estimated uncertainty when evaluated systematically across chemicals

Using *in vivo* Data to Evaluate RTK



- When we compare the C_{ss} predicted from *in vitro* HTTK with *in vivo* C_{ss} values determined from the literature we find limited correlation (R^2 ~0.34)
- The dashed line indicates the identity (perfect predictor) line:
  - Over-predict for 65
  - Under-predict for 22
- The white lines indicate the discrepancy between measured and predicted values (the residual)



Wambaugh et al. (2015)

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Predicting When RTK Will Work

- We can use computer algorithms to analyze chemical descriptors to try to predict when the residual will be small
- Factors included are:
  - Physico-chemical properties
    - Log(Kow), molecular weight, acid/base association constants (pKa), general pharmaceutical or perfluorinated compound classification
  - *In vitro* HTTK data
    - Plasma protein binding (F_{up}) and hepatic clearance
  - Active chemical transport
    - Use quantitative structure activity relationships (QSARs) to predict likelihood each compound is a substrate for 17 different transporters (From Alexander Sedykh and Alex Tropsha (UNC) and Sieto Bosgra (TNO))

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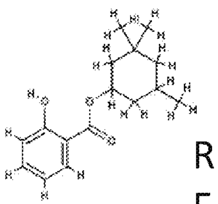
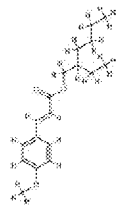
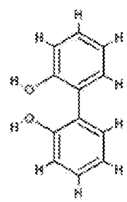
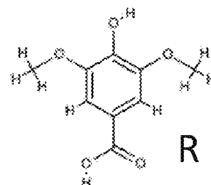
Predicting RTK Errors

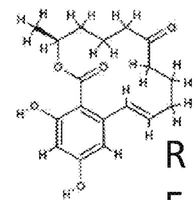
- The higher the C_{ss}, the lower the oral equivalent dose
- Ideally the residuals (difference between the literature value and the prediction) are small or $R \equiv C_{ss}^{lit.}/C_{ss}^{pred.} \approx 1$
- If a residual is large, we would prefer to over-predict C_{ss} to be conservative, *i.e*. $R < 1$


$R = 5$
$F_{up} = 0.5$


$R = 1.02$
$F_{up} = 0.06$

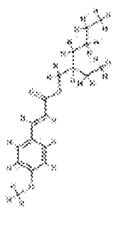

$R = 10$
$F_{up} = 0.08$

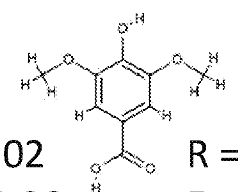

$R = 0.5$
$F_{up} = 0.04$

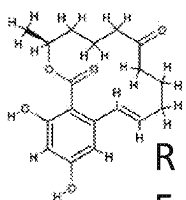

$R = 0.9$
$F_{up} = 0.02$

$F_{up} < 0.11$

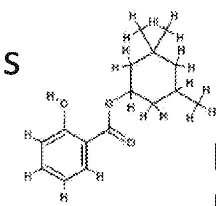
No

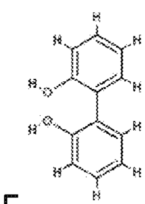

$R = 1.02$
$F_{up} = 0.06$


$R = 0.5$
$F_{up} = 0.04$


$R = 0.9$
$F_{up} = 0.02$

YEs


$R = 5$
$F_{up} = 0.5$


$R = 10$
$F_{up} = 0.08$

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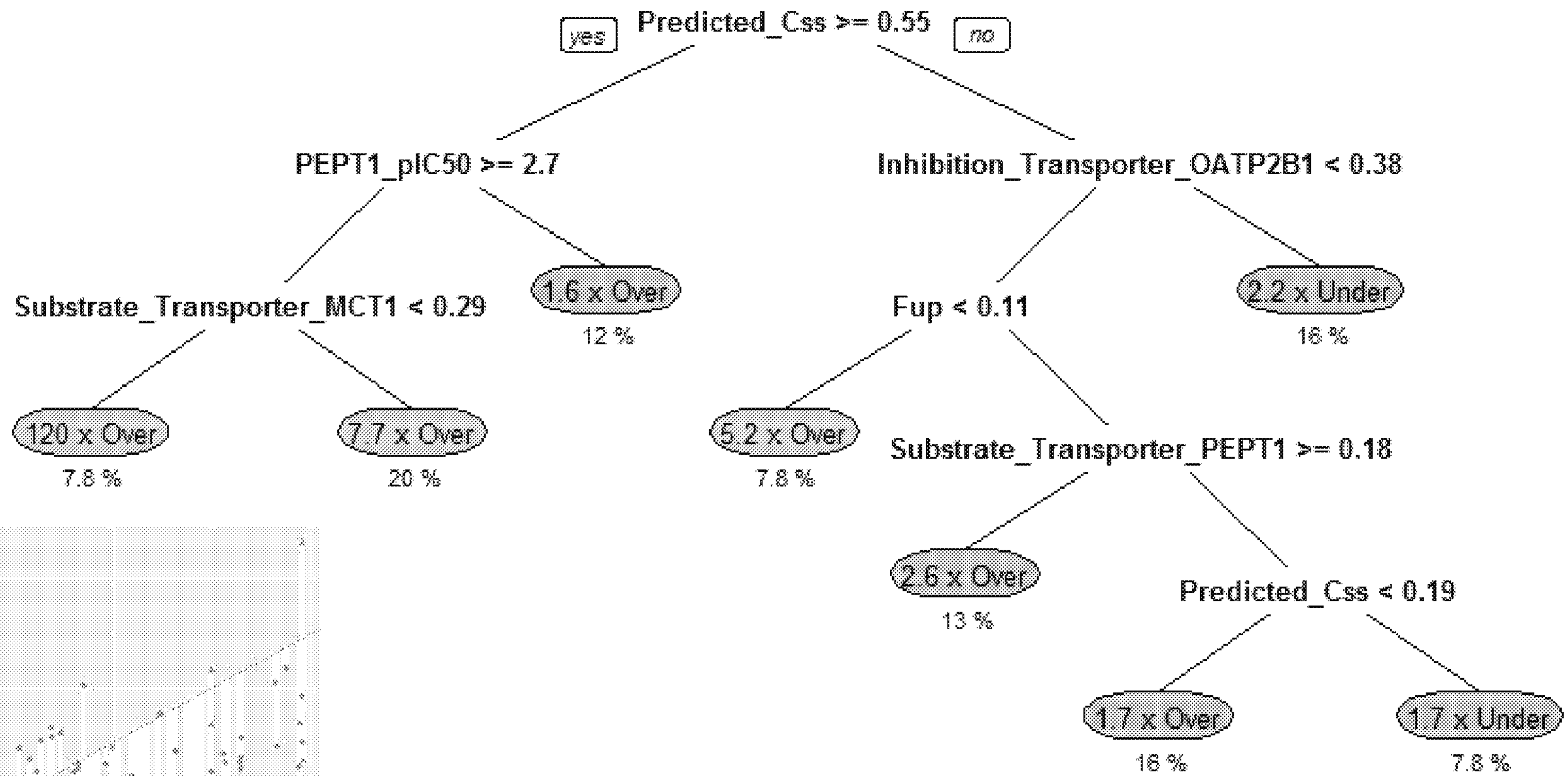
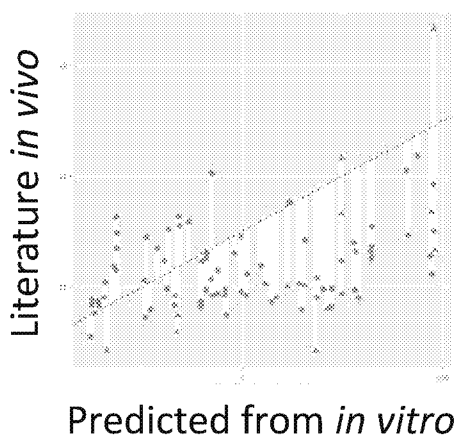
Predicting HTTK Errors





- If the predicted C_{ss} overestimates the literature value, the necessary exposure (i.e., equivalent dose) predicted with RTK will be lower
  - This is a conservative error for reverse dosimetry
- Worry about cases where we significantly underestimate necessary exposure

Wambaugh et al. (2015)

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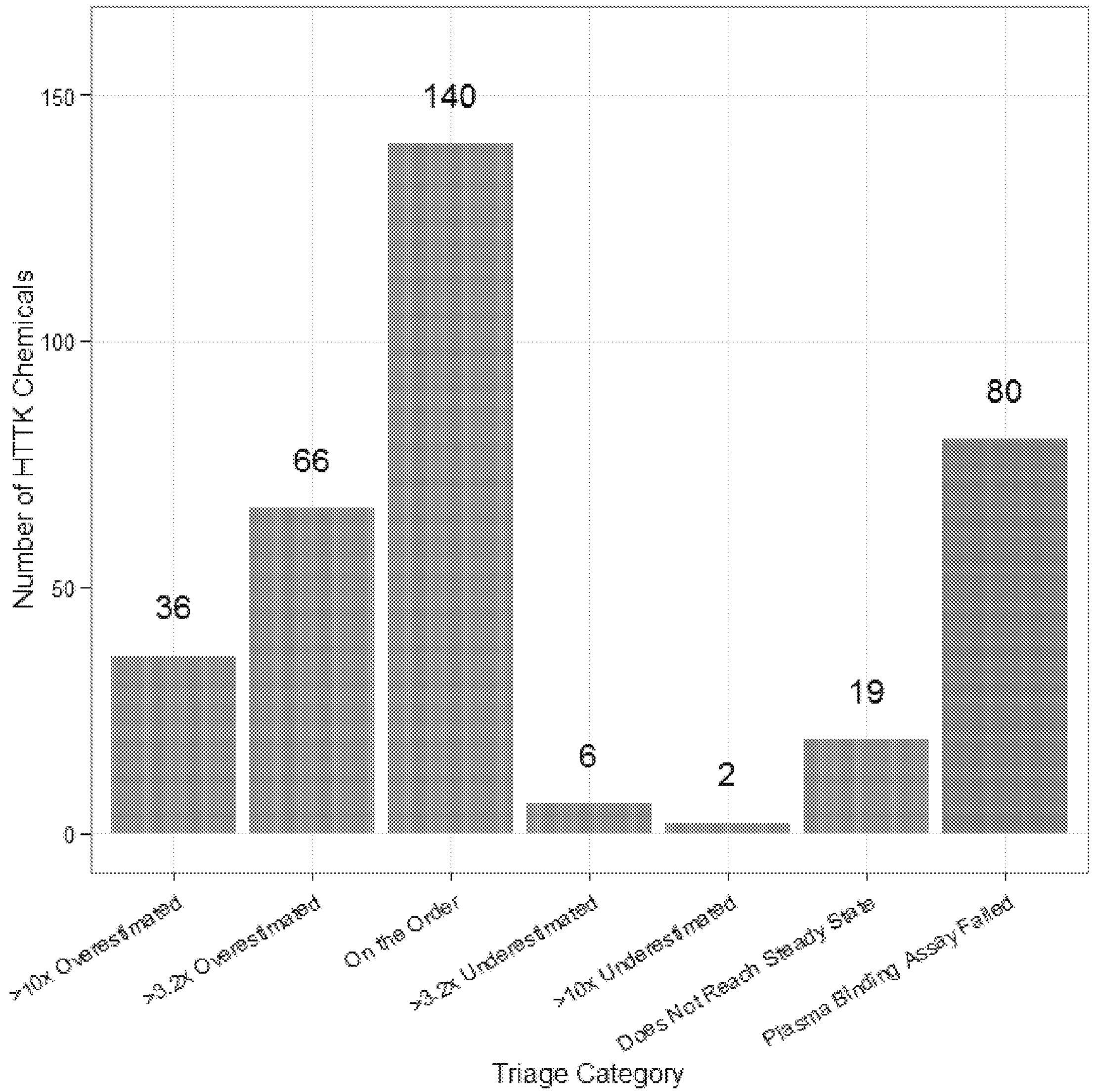
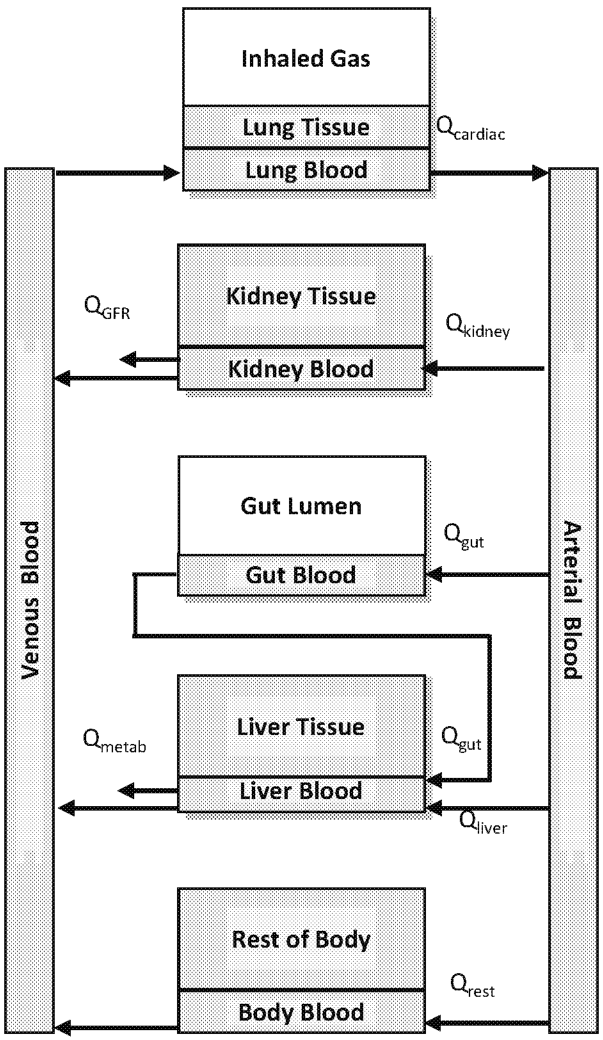
Toxicokinetic Triage

- Through comparison to *in vivo* data, a cross-validated (random forest) predictor of success or failure of HTTK has been constructed
- Add categories for chemicals that do not reach steady-state or for which plasma binding assay fails
- All chemicals can be placed into one of seven confidence categories



Wambaugh et al. (2015)

A General Physiologically-based Toxicokinetic (PBTK) Model



- "httk" also includes a generic PBTK model
- Some tissues (e.g. arterial blood) are simple compartments, while others (e.g. kidney) are compound compartments consisting of separate blood and tissue sections with constant partitioning (i.e., tissue specific partition coefficients)
- Exposures are absorbed from reservoirs (gut lumen)
- Some specific tissues (lung, kidney, gut, and liver) are modeled explicitly, others (e.g. fat, brain, bones) are lumped into the "Rest of Body" compartment.
- Blood flows move the chemical throughout the body. The total blood flow to all tissues equals the cardiac output.
- The only ways chemicals "leaves" the body are through metabolism (change into a metabolite) in the liver or excretion by glomerular filtration into the proximal tubules of the kidney (which filter into the lumen of the kidney).

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Basic PK Statistics Examples

```
library(httk)
#A Function to get PK summary statistics from the PBPK model:
help(calc_stats)
# 28 day human study (20 mg/kg/day) for Abamectin:
calc_stats(days=28,chem.name="bisphenol a", dose=20)
    Human plasma concentrations returned in uM units.
    AUC is area under plasma concentration curve in uM * days units with Rblood2plasma = 0.79 .
    $AUC
    [1] 44.82138
    $peak
    [1] 23.16455
    $mean
    [1] 1.600764
# Units default to µM but can use mg/L:
calc_stats(days=28,chem.name="bisphenol a", dose=20,output.units="mg/L")
# Same study in a mouse:
calc_stats(days=28,chem.name="bisphenol a", dose=20,species="mouse")
```


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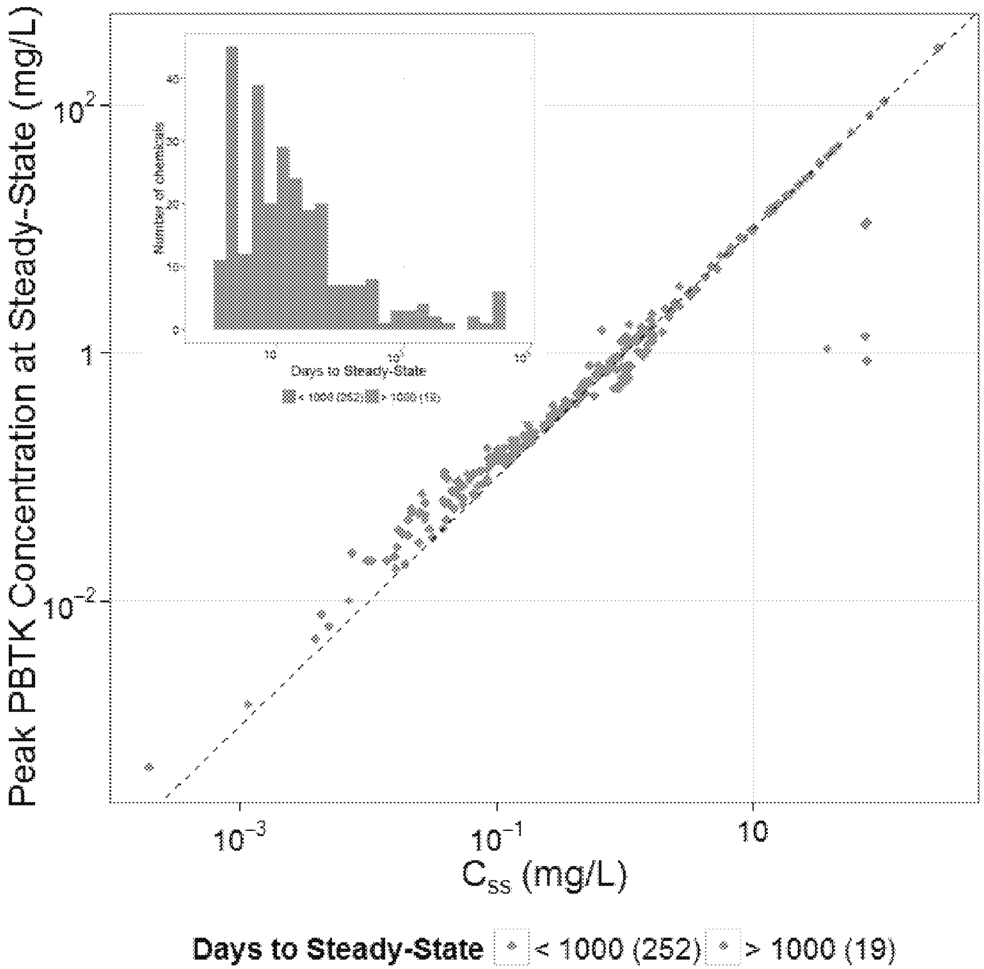
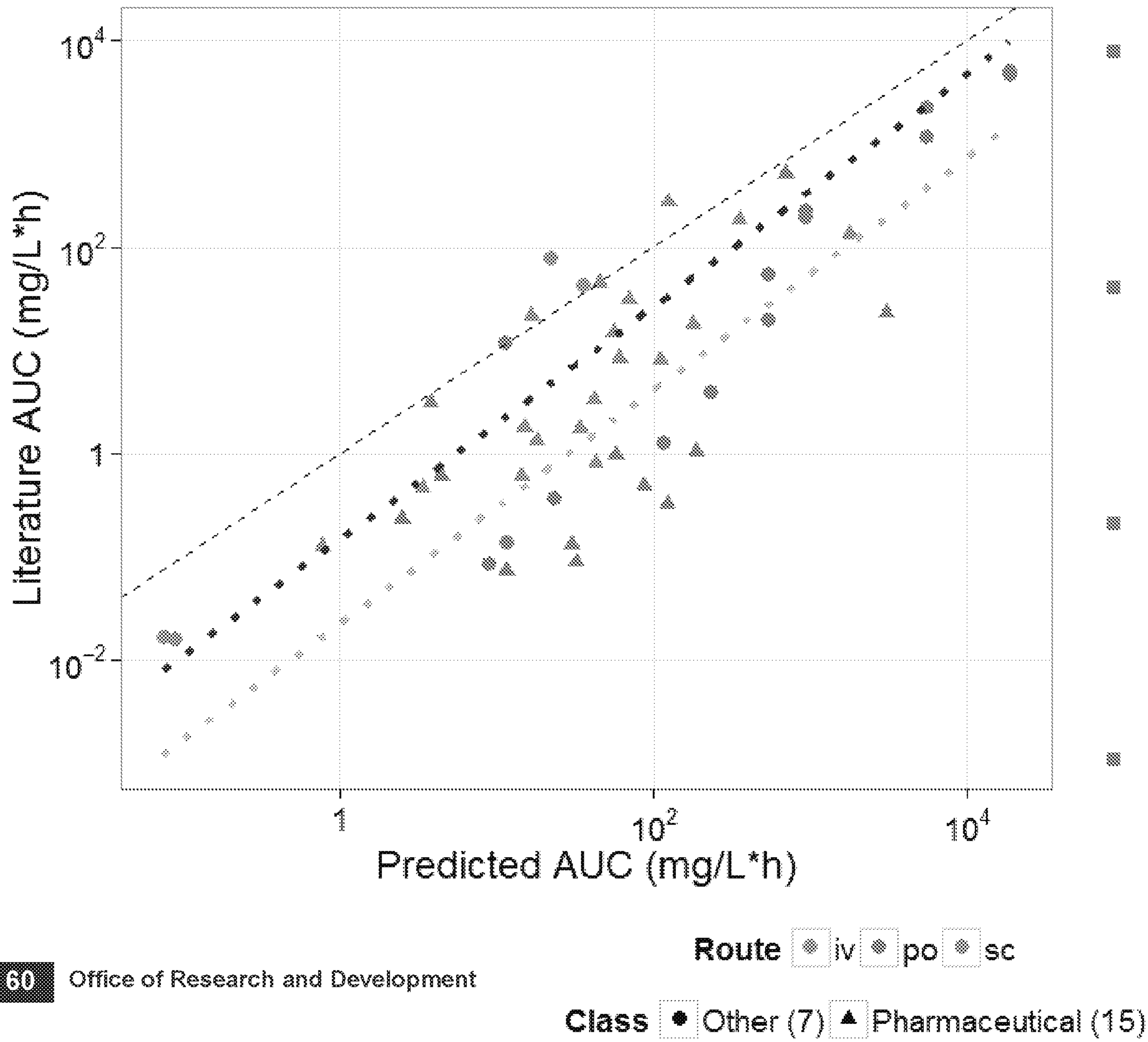
Peak Concentration vs. C_{ss}



- Peak serum concentrations from the HTPBTK model are compared against the steady-state concentration predicted by the three compartment model for a constant infusion exposure (as in Wetmore *et al.* 2012)
- The dashed, identity (1:1) line indicates that for most compounds the peak concentrations are very similar to C_{ss}

Wambaugh et al. (2015)

Evaluating *In Vitro* PBTK Predictions with *In Vivo* Data



- PBTK predictions for the AUC (time integrated plasma concentration or Area Under the Curve)
- *in vivo* measurements from the literature for various treatments (dose and route) of rat.
- Predictions are generally conservative – *i.e.*, predicted AUC higher than measured
- Oral dose AUC ~6.4x higher than intravenous dose AUC

Wambaugh et al. (2015)

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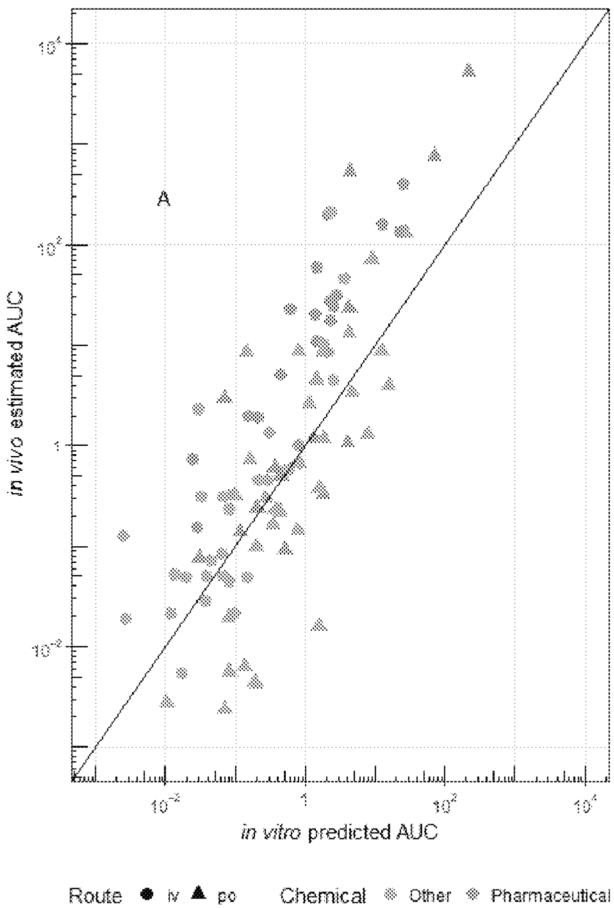
Analyzing New *In Vivo* Data (Rat)



- Oral and *iv* studies for 26 ToxCast compounds
  - Collaboration with NHEERL (Mike Hughes and Jane Ellen Simmons)
  - Additional work by Research Triangle Institute (Tim Fennell)
- Can estimate
  - Fraction absorbed
  - Absorption Rate
  - Elimination Rate
  - Volume of Distribution

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Analyzing New *In Vivo* Data (Rat)

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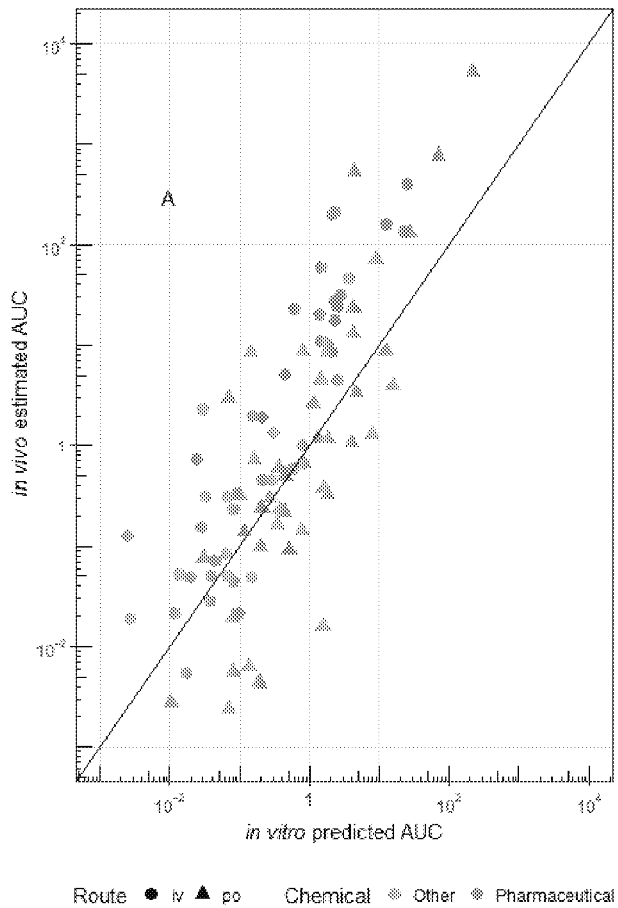

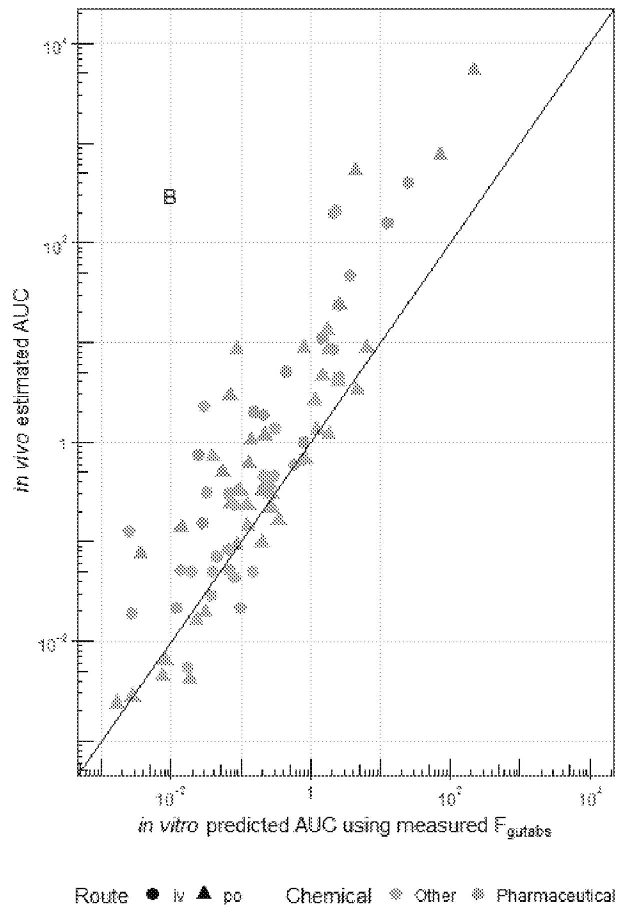

- Oral and *iv* studies for 26 ToxCast compounds
  - Collaboration with NHEERL (Mike Hughes and Jane Ellen Simmons)
  - Additional work by Research Triangle Institute (Tim Fennell)
- Can estimate
  - Fraction absorbed
  - Absorption Rate
  - Elimination Rate
  - Volume of Distribution

Cyprotex (ToxCast) is now measuring bioavailability (CACO2) for many HTTK chemicals

Population simulator for HTTK

Correlated Monte Carlo sampling of physiological model parameters

- Body weight
- Tissue masses
- Tissue blood flows
- GFR (kidney)
- Hepatocellularity

Source of data: CDC NHANES

National Health and Nutrition Examination Survey

Large, ongoing CDC survey of US population: demographic, body measures, medical exam, biomonitoring (health and exposure), …

Designed to be representative of US population according to census data

Data sets publicly available (http://www.cdc.gov/nchs/nhanes.htm)

Ring et al. (*in press*)

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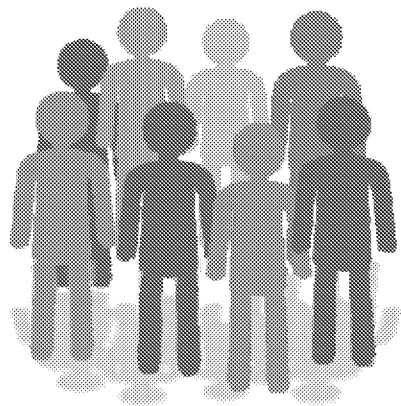
Population simulator for HTTK

Sample
NHANES quantities

Sex
Race/ethnicity
Age
Height
Weight
Serum creatinine



Regression equations from literature (+ residual marginal variability)

Predict
physiological quantities

Tissue masses
Tissue blood flows
GFR (kidney function)
Hepatocellularity

(Similar approach used in SimCYP [Jamei et al. 2009], GastroPlus, PopGen [McNally et al. 2014], P3M [Price et al. 2003], physB [Bosgra et al. 2012], etc.) Ring et al. (*in press*)

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Generating demographic subgroups

User can specify....	Default if not specified
Age limits	0-79 years
Sex (# males, # females)	NHANES proportions
Race/ethnicity (5 NHANES categories)	NHANES proportions
BMI/weight categories	NHANES proportions

- NHANES quantities sampled from appropriate *conditional* distribution (given specifications)
  - Physiological parameters predicted accordingly

Ring et al. (*in press*)

NHANES Demographic Examples

```
library(httk)

# Oral equivalent (mg/kg/day) for in vitro activity of 1 µM for Acetochlor
calc_mc_oral_equiv(1,chem.cas="34256-82-1",method="dr")

# Oral equivalent (mg/kg/day) for NHANES "Mexican American" Population
calc_mc_oral_equiv(1,chem.cas="34256-82-1",method="dr", reths = "Mexican American")

# Oral equivalent (mg/kg/day) for NHANES "Mexican American" Population aged 18-25 years
calc_mc_oral_equiv(1,chem.cas="34256-82-1",method="dr",agelim_years=c(18,25),reths =
"Mexican American")

# Probably too few individuals in NHANES for direct resampling ("dr") so use virtual individuals
("vi") resampling method:
calc_mc_oral_equiv(1,chem.cas="34256-82-1",method="vi",agelim_years=c(18,25),reths =
"Mexican American")
```

Can also specify gender, weight categories, and kidney function

Ring et al. (*in press*)

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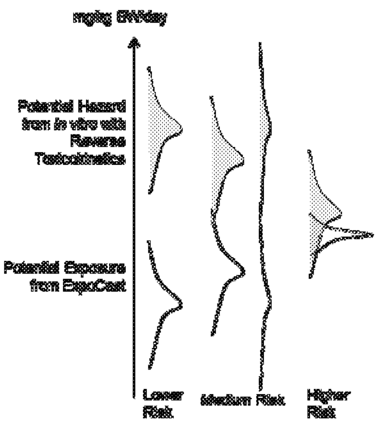
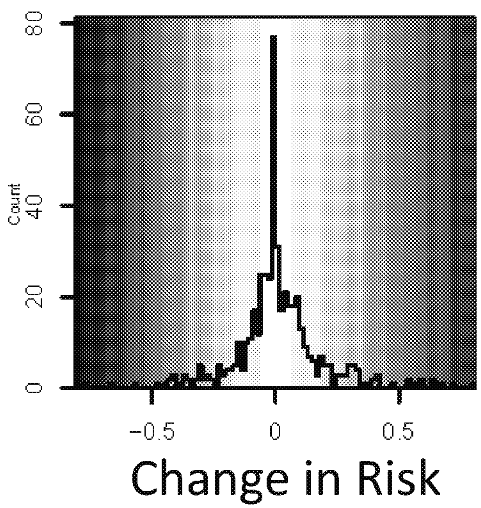
Life-stage and Demographic Specific Predictions

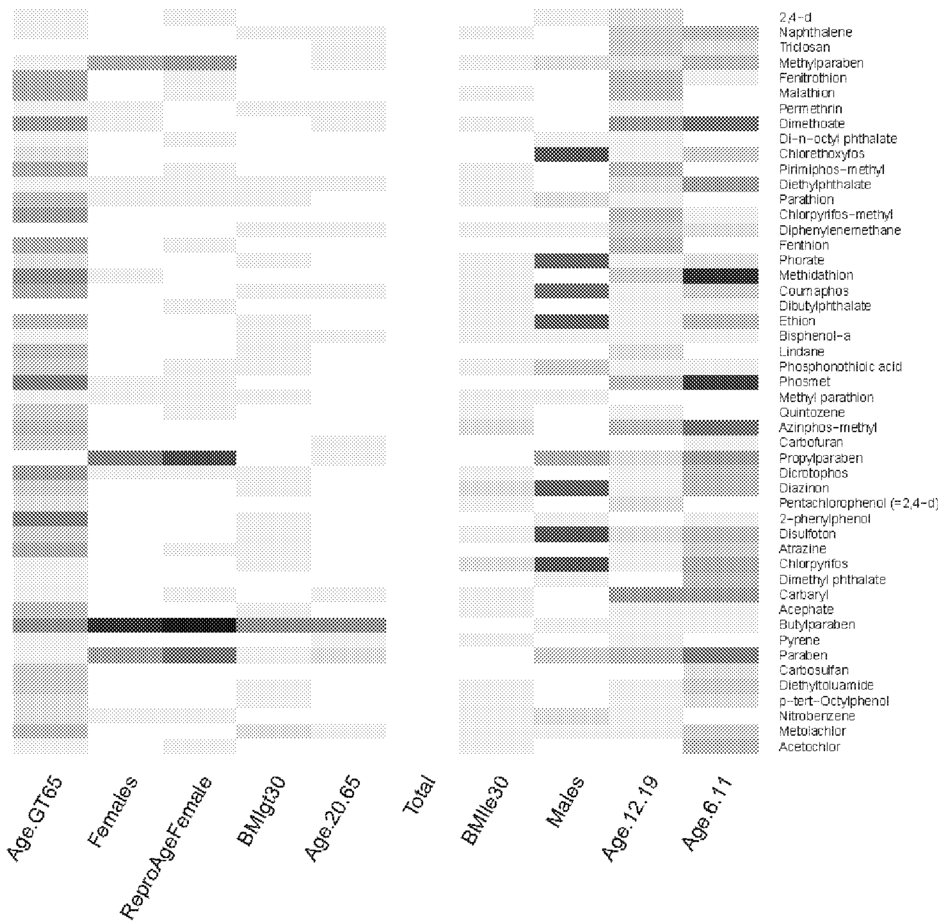
- Wambaugh *et al.* (2014) predictions of exposure rate (mg/kg/day) for various demographic groups

- Can use HTTK to calculate margin between bioactivity and exposure for specific populations





Change in Activity:Exposure Ratio



Ring et al. (*in press*)

 Office of Research and Development

United States Environmental Protection Agency

Version history for "httk"

The publicly available R package contains code and data that has been part of peer-reviewed publications (Old versions are archived)

- Version 1.1 accompanied "Toxicokinetic Triage for Environmental Chemicals" Wambaugh et al. (2015) Tox. Sci.
- Version 1.2 accompanied "httk: R Package for High-Throughput Toxicokinetics" Pearce et al., Journal of Statistical Software (*in press*)
- Version 1.3 accompanied "Incorporating High-Throughput Exposure Predictions with Dosimetry-Adjusted *In Vitro* Bioactivity to Inform Chemical Toxicity Testing" Wetmore et al., (2015) Tox. Sci.
- Version 1.4 addressed comments for acceptance of Pearce et al. (*in press, J. Stat. Soft.*)
- Version 1.5 accompanied "Identifying populations sensitive to environmental chemicals by simulating toxicokinetic variability," Ring et al. (*in press, Env. International*)
- Version 1.6 accompanied "Evaluation and Calibration of High-Throughput Predictions of Chemical Distribution to Tissues," Pearce et al. (*submitted*)

- Subsequent version numbers will be assigned as papers are accepted on:
    - Gestational model (Kapraun)
    - Inhalation exposure (Evans and Pearce)
    - New human data from Cyprotex (Wambaugh and Wetmore)
    - New rat data and revised IVIVE model (Honda)
    - More flexible PBPK model (Pearce)

Lead programmer Robert Pearce

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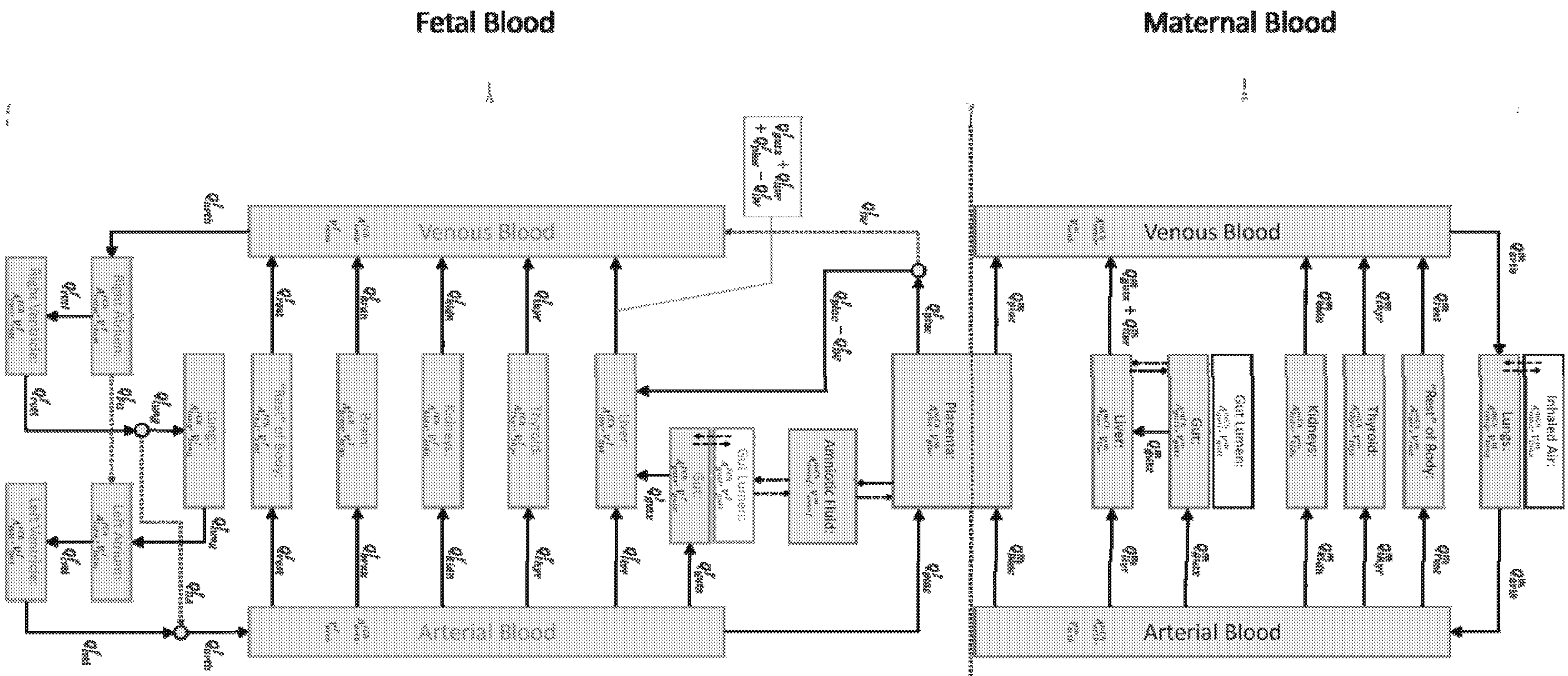
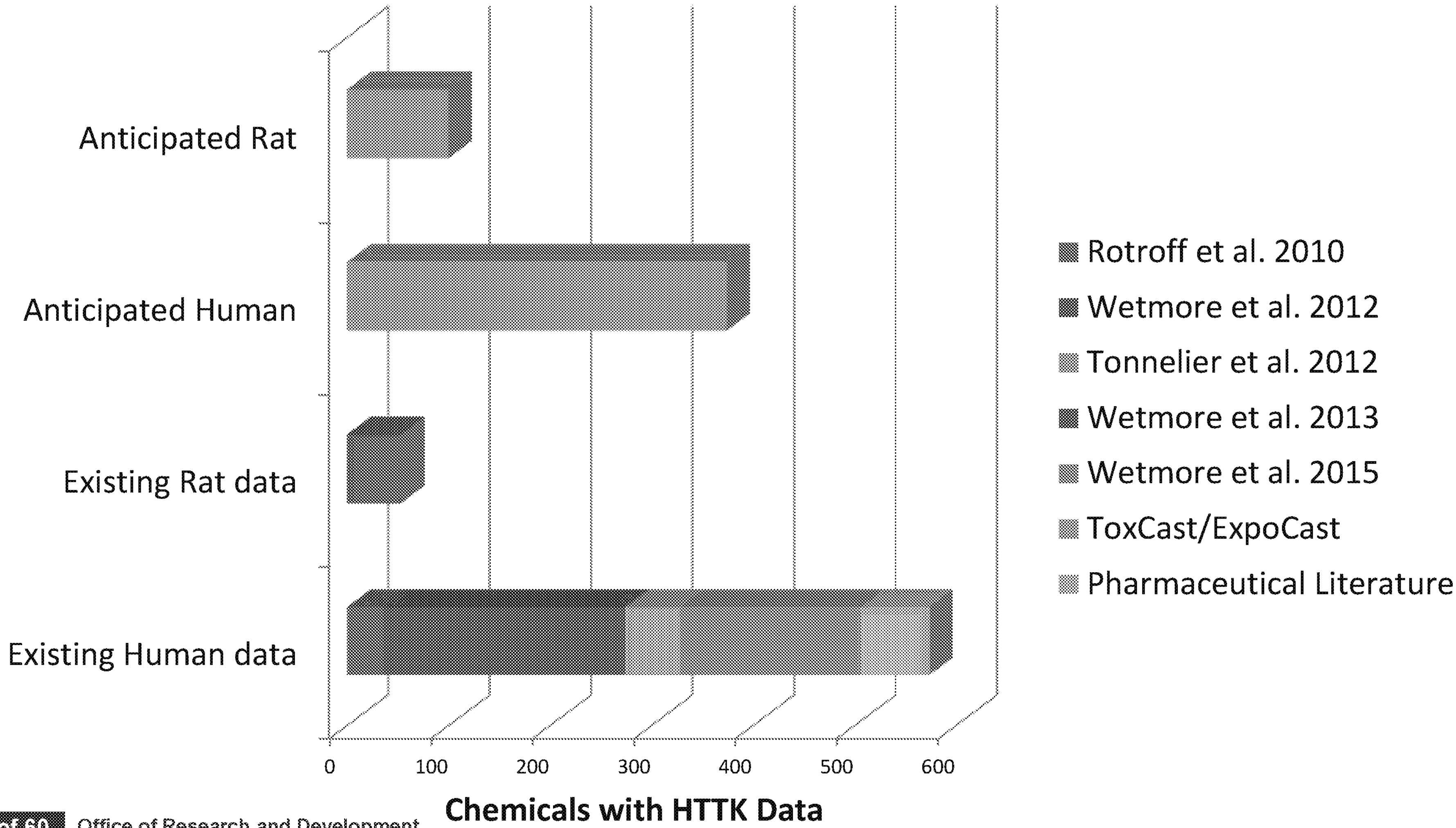
Gestational Version of PBTK model Under Development



New httk package model that allows fetal tissue concentration predictions for all PBTK chemcials

Kapraun et al., (in preparation)

Chemicals with HTTK Data



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Does My Chemical Have HTTK Data?

Is a chemical available?

```
> "80-05-7" %in% get_cheminfo()
[1] TRUE
```

```
> library(httk)
> get_cheminfo()
 [1] "2971-36-0"  "94-75-7"    "94-82-6"    "90-43-7"    "1007-28-9"
 [6] "71751-41-2" "30560-19-1" "135410-20-7" "34256-82-1" "50594-66-6"
[11] "15972-60-8" "116-06-3"   "834-12-8"    "33089-61-1" "101-05-3"
[16] "1912-24-9"  "86-50-0"    "131860-33-8" "22781-23-3" "1861-40-1" ...
> get_cheminfo(info="all")
```

All data on chemicals A, B, C

```
subset(get_cheminfo(info
="all"),Compound%in%c(
"A","B","C"))
```

Compound	CAS	logP	pKa_Accept	pKa_Donor	MW	Human.Clint	Human.Clint.pValue	Human.Funbound.plasma	DSSTox_Substance_Id	Structure_Formula	Substance_Type	
2,4-d	94-75-7	2.81	<NA>	2.81	221.03	0	0.149	0.04	DTXSID0020442	C8H6Cl2O3	Single	Compound
2,4-db	94-82-6	3.53	<NA>	4.5	249.09	0	0.104	0.01	DTXSID7024035	C10H10Cl2O3	Single	Compound
2-phenylphenol	90-43-7	3.09	<NA>	10.6	170.211	2.08	0.164	0.04	DTXSID2021151	C12H10O	Single	Compound
6-desisopropylatrazine	1007-28-9	1.15	1.59	<NA>	173.6	0	0.539	0.46	DTXSID0037495	C5H8ClN5	Single	Compoun

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Summary

- Toxicokinetics (TK) provides a bridge between HTS and HTE by predicting tissue concentrations due to exposure
- High Throughput (HTTK) methods developed for pharmaceuticals have been adapted to environmental testing
- A primary application of HTTK is "Reverse Dosimetry" or RTK
  - Can infer daily doses that produce plasma concentrations equivalent to the bioactive concentrations,
  - **But:** We must consider "domain of applicability"
- New R package "httk" freely available on CRAN allows statistical analyses to identify strengths and weaknesses
  - All HTTK models and data made public upon peer-reviewed publication

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The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA

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References

- Bosgra, S ., et al. "An improved model to predict physiologically based model parameters and their inter-individual variability from anthropometry." Critical reviews in toxicology 2012;42:751-767
- Filer, Dayne L., et al. "tcpl: The ToxCast Pipeline for High-Throughput Screening Data." Bioinformatics (2016): btw680.
- Howgate, E., et al. "Prediction of in vivo drug clearance from in vitro data. I: impact of inter-individual variability" Xenobiotica 2006;36:473-497
- Israili and Dayton "Human Alpha-1-Glycoprotein and Its Interactions with Drugs" Drug metabolism reviews 2001;33:161-235
- Jamei, et al. "The Simcyp® population-based ADME simulator." Expert opinion on drug metabolism & toxicology 2009b;5:211-223
- Johnson, et al. "Prediction of the clearance of eleven drugs and associated variability in neonates, infants and children." Clinical pharmacokinetics (2006)
- Judson, R. S., et al., (2010) "In Vitro Screening of Environmental Chemicals for Targeted Testing Prioritization: The ToxCast Project. Environmental Health Perspectives 118(4), 485-492.
- Judson, R. S., et al., (2011). Estimating Toxicity-Related Biological Pathway Altering Doses for High-Throughput Chemical Risk Assessment. Chemical Research in Toxicology 24(4), 451-462
- McNally, et al., "PopGen: a virtual human population generator." Toxicology 2014
- Park, Youngja, H., et al. "High-performance metabolic profiling of plasma from seven mammalian species for simultaneous environmental chemical surveillance and bioeffect monitoring." Toxicology 295:47-55 (2012)
- Pearce, Robert, et al. "httk: R Package for High-Throughput Toxicokinetics." Journal of Statistical Software, *in press.*
- Price et al., "Instructions for Use of Software Physiological Parameters for PBPK Modeling Version 1.3 (P3MTM 1.3)." 2003
- Ring , Caroline, et al., "Identifying populations sensitive to environmental chemicals by simulating toxicokinetic variability", Environment International, *in press*
- Rotroff, Daniel, et al., (2010) "Incorporating human dosimetry and exposure into high-throughput in vitro toxicity screening." Tox. Sciences 117(2), 348-58
- Routledge, P., "The plasma protein binding of basic drugs. British journal of clinical pharmacology 1986;22:499-506
- Schmidt, Charles W. "TOX 21: new dimensions of toxicity testing." Environ Health Perspect 117.8 (2009): A348-A353.
- Shibata, et al., (2002). Prediction of Hepatic Clearance and Availability by Cryopreserved Human Hepatocytes: An Application of Serum Incubation Method. Drug Metabolism and Disposition 30(8), 892-896
- Strope, Cory L., et al., "High-Throughput in-silico prediction of ionization equilibria for pharmacokinetic modeling", *in ORD clearance.*
- Wambaugh, John F., et al. "Dosimetric anchoring of in vivo and in vitro studies for perfluorooctanoate and perfluorooctanesulfonate." *Toxi.Sciences* (2013)
- Wambaugh, John F., et al. "High Throughput Heuristics for Prioritizing Human Exposure to Environmental Chemicals." *Env. science & technology* (2014).
- Wambaugh, John F., et al. "Toxicokinetic triage for environmental chemicals." Toxicological Sciences (2015): kfv118.
- Wang, Y.-H. (2010). "Confidence Assessment of the Simcyp Time-Based Approach and a Static Mathematical Model in Predicting Clinical Drug-Drug Interactions for Mechanism-Based CYP3A Inhibitors." Drug Metabolism and Disposition 38(7), 1094-1104
- Waters, N. J., et al. (2008). "Validation of a rapid equilibrium dialysis approach for the measurement of plasma protein binding." Journal of Pharmaceutical Sciences 97(10), 4586-4595
- Wetmore, Barbara A., et al. "Integration of dosimetry, exposure and high-throughput screening data in chemical toxicity assessment." *Tox. Sciences* (2012)
- Wetmore, Barbara A., et al. "Relative Impact of Incorporating Pharmacokinetics on Predicting In Vivo Hazard and Mode of Action from High-Throughput In Vitro Toxicity Assays." Toxicological Sciences 132(2), 327-346
- Wetmore, Barbara A., et al., "Incorporating population variability and susceptible subpopulations into dosimetry for high-throughput toxicity testing. Toxicological sciences 2014;142:210-224
- Wetmore, Barbara A., et al. "Incorporating High-Throughput Exposure Predictions with Dosimetry-Adjusted In Vitro Bioactivity to Inform Chemical Toxicity Testing." *Toxicological Sciences* 148.1 (2015): 121-136.
- Yasuda, et al., "The role of ethnicity in variability in response to drugs: focus on clinical pharmacology studies." Clinical Pharmacology & Therapeutics 2008;84:417-423
- Yoon, M., et al. (2014). "Evaluation of simple in vitro to in vivo extrapolation approaches for environmental compounds." Toxicology in Vitro 28(2), 164-170,